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Toxicity, Bioconcentration, and Metabolism of Five Herbicides in Freshwater Fish

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16. ABSTRACT

Toxicological studies were conducted in two areas: (1) the toxicity, bioconcentration potential and metabolism of five herbicides in fish; and (2) the toxicity and/or metabolism of priority pollutants and related chemicals in various aquatic organisms.

The test herbicides included alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide], bromacil (5-bromo-3-sec-butyl-6-methyluracil), dinoseb [2-(sec-butyl)-4,6-dinitrophenol], diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], and propanil (3,4-dichloropropionanilide). Acute toxicity (through 192 hr), early life-stage toxicity (58-64 day), and bioconcentration studies were conducted with fathead minnows (Pimephales promelas) in Lake Superior water. Herbicide metabolism was investigated in rainbow trout (Salmo gairdneri) both in vivo and in vitro.

Twenty-two chemicals from the EPA priority pollutant list were studied for their acute and/or chronic toxicity to selected freshwater organisms. These included 1,2-dichloroethane, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, tetrachloroethylene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, hexachlorobenzene, hexachlorobutadiene, di-n-butylphthalate, pentachlorophenol, heptachlor, chlordane, toxaphene, arsenic^{+3}, chromium^{+6}, lead^{+2}, mercury^{+2}, nickel^{+2}, silver^{+1}, selenium^{+4}, and cyanide. Freshwater species tested included the fathead minnow, rainbow trout, bluegill sunfish (Lepomis macrochirus), flagfish (Jordanella floridae), Daphnia magna, scud (Gammarus pseudolimnaeus), midge (Tanytarsus dissimilis) and green alga (Selenastrum capricornutum). Toxicity tests were also conducted with pentachloroethane, hexachloroethane, 1,2,4-trichlorobenzene, pentachlorobenzene, methanol and dimethylformamide. The uptake by fish of di-n-butylphthalate from water, its metabolism and elimination were investigated. Comparative metabolism of 1,1,2-trichloroethane, chlorobenzene, 1,1,2-trichloroethylene, chloroform, and carbon tetrachloride was studied in rainbow trout and Daphnia.

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ABSTRACT

Acute and early life-stage toxicities were determined for the herbicides alachlor, bromacil, dinoseb, diuron, and propanil with the fathead minnow (*Pimephales promelas*). Uptake, bioconcentration potential, and elimination of ^{14}C-labeled herbicides were studied in the same species. *In vivo* metabolism of radiolabeled herbicides was determined with rainbow trout (*Salmo gairdneri*) and fathead minnows as test organisms. *In vitro* metabolism was studied with rainbow trout liver homogenates.

LC_{50} values at 96 hr in Lake Superior water for 30-day old fathead minnows were: alachlor (5.0 $mg \cdot L^{-1}$), bromacil (182 $mg \cdot L^{-1}$), dinoseb (0.7 $mg \cdot L^{-1}$), diuron (14.2 $mg \cdot L^{-1}$), and propanil (8.6 $mg \cdot L^{-1}$). Mortality readings were made at intervals up to 192 hr to obtain mortality curves for each herbicide.

The "no-effect" estimate for a 60-day post-hatch exposure of fathead minnows to alachlor was between 0.52 and 1.10 $mg \cdot L^{-1}$, based upon fish weight and length. A "no-effect" concentration range for a 60-day exposure to bromacil could not be determined from the data, but was less than 1.0 $mg \cdot L^{-1}$. The 60-day "no-effect" estimate for dinoseb was between 14.5 and 48.5 $\mu g \cdot L^{-1}$, based upon the two most sensitive parameters, fish survival and weight. The 60-day "no-effect" estimate for diuron was between 33.4 and 78.0 $\mu g \cdot L^{-1}$ based upon two significantly affected parameters, abnormal fry development and fish survival through 60 days. A 54-day "no-effect" estimate for propanil was between 0.4 and 0.6 $\mu g \cdot L^{-1}$ based upon fish length and dry weight as the two

FOREWORD

Pesticides are applied to land and water in high tonnage volumes each year. These chemicals must be environmentally safe as well as effective. The United States Environmental Protection Agency researches and reviews pesticides that are currently registered and those which are under registration consideration for their environmental safety.

This report contains the results of toxicity, bioconcentration, and metabolism studies with five registered herbicides and fish. This information will assist the Office of Pesticide Programs in its evaluation of these herbicides for their possible impacts upon the environment.

Norbert Jaworski, Ph. D.
Director
Environmental Research Laboratory
Duluth, MN

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This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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TOXICITY, BIOCONCENTRATION, AND METABOLISM OF FIVE HERBICIDES IN FRESHWATER FISH

by

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most sensitive parameters.

Fathead minnow mean bioconcentration factors for ^{14}C parent herbicide equivalents were: alachlor (45.8 x), bromacil (3.2 x), dinoseb (62.8 x), diuron (150.4 x), and propanil (90.2 x). Percent parent compound at the end of the uptake phase for alachlor, dinoseb, diuron, and propanil were 13.2, 2.3, 1.3, and 1.8%, respectively. This was not determined for bromacil. Parent herbicide bioconcentration factors were: alachlor (6.0 x), dinoseb (1.4 x), diuron (2.0 x), and propanil (1.6 x).

Rainbow trout injected with ^{14}C-labeled herbicides readily metabolized all five compounds. From 75 to 98% of the injected doses were excreted within 24 hrs. Metabolites of the herbicides accumulated in the bile to varying degrees - from 1% (bromacil) to 22% (propanil) of the injected dose. GC/MS characterization of extracts from diuron exposed trout revealed the presence of 3,4-dichloroaniline and demethylated products. A propanil metabolite was identified as either 3',4'-dichloro-2-hydroxypropionanilide or 3',4'-dichloro-3-hydroxypropionanilide.

<u>In vitro</u> binding of herbicides to rainbow trout liver macromolecules occurred for all five compounds. Alachlor, bromacil, dinoseb, diuron, and propanil had protein binding values of 75.2, 3.6, 13.2, 6.7, and 2.3 nanomoles herbicide·gm^{-1} of protein, respectively. The binding was not dependent on NADPH, was not altered by the addition of glutathione, and was not altered by the substitution of heat-inactivated microsomes for active microsomes. The binding was apparently due to the affinity of the parent herbicides for protein and other macromolecules, and was not dependent on mixed-function oxidase activity.

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SECTION I

INTRODUCTION

Contamination of non-target organisms, including man, by pesticides is of common occurrence. Watersheds throughout the world are annually exposed to pesticides, some very intensively. Certain pesticides, applied terrestrially, enter aquatic ecosystems. Additionally, selected pesticides are applied directly to aquatic ecosystems for control of undesirable plant and animal species. These exposures create the potential for stress at all trophic levels in such ecosystems.

The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) of 1947 established the registration process for pesticides used in this country. The Act was amended by the Federal Environmental Pesticide Control Act of 1972 to more strictly control pesticide manufacture, sale and use. In 1975 the U.S. Environmental Protection Agency established the "Rebuttable Presumption Against Registration" (RPAR) process[1] to review pesticide usage patterns and to determine whether specific pesticides pose substantial questions of safety, subsequently necessitating denial or cancellation of registrations under this Act. As a result of these reviews, some pesticides will undoubtedly not be reregistered and needs for alternative pesticides will develop. The five herbicides of this study have been produced and marketed for several years, and are being considered as alternative pesticides.

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[1] Federal Register, Vol. 40, Sec. 162.11, pp. 28268, 32329, 42746.

Alachlor is a pre-emergence herbicide introduced by Monsanto Company in 1967 for control of various annual grasses and certain broadleaf weeds such as nutsedge (Agriculture Canada, 1973). It is commonly used for weed control in corn and soybeans.

Bromacil, manufactured by DuPont, was first used publicly in 1963 (U.S. EPA, 1974). Its primary uses are for control of perennial grasses, residual weeds, and brush at industrial sites, utility rights-of-way, railroads, and ditch or canal banks (U.S. EPA, 1974).

Dinoseb is manufactured in the United States by Dow Chemical Company (Agriculture Canada, 1973). It was first described as a weed killer in 1945, and was the most toxic of a number of dinitro substituted phenols tested for phytotoxicity (Crafts, 1945). Dinoseb ammonium salt is used as a post-emergence, selective spray in flax, beans, peas, leek, potatoes, coffee, vineyards and orchards; and as a dessicant in potatoes and legume crops grown for seed (Crafts, 1975). Dinoseb as the parent phenol in oil is used as a general contact spray; and the alkanolamine salt is used to kill germinating seeds and as early postemergence and directed sprays (Crafts, 1975).

Diuron, manufactured by E.I. DuPont de Nemours & Company, was introduced as an experimental compound in 1951, registered for industrial weed control in 1953 and for agricultural use in 1954 (Johnson and Julin, 1974). Diuron has been used for many years for selective control of annual grasses and broadleaf weeds in apple, pineapple, sugarcane, and cotton (Majka and Lavy, 1977). It has also been extensively studied and variously used for aquatic weed control, although it has not been registered for aquatic use (Johnson and Julin, 1974).

Propanil is a postemergence amide herbicide used in the United States to control barnyardgrass and other weeds in rice (U.S. EPA, 1974; Smith, 1965).

Economically, it is the most important acylanilide herbicide (Hsia and Burant, 1979).

The present study was undertaken to determine the acute and early life-stage toxicities, bioconcentration potential, and metabolism of these five herbicides in freshwater fish. Toxicity tests and bioconcentration studies were conducted with the fathead minnow (Pimephales promelas). Metabolism studies utilized both the fathead minnow and rainbow trout (Salmo gairdneri) as test species.

SECTION II

CONCLUSIONS

96 Hr LC_{50} values (95% confidence intervals in parentheses) for fathead minnows exposed to alachlor, bromacil, dinoseb, diuron, and propanil were 5.0 (4.5 -5.6), 182 (177-188), 0.7 (0.6-0.7), 14.2 (13.4-15.0), and 8.6 (7.7-9.5) $mg \cdot L^{-1}$, respectively.

Based upon one or more of the parameters of dead and abnormal fry, juvenile fish survival, and juvenile fish growth in early life-stage tests with fathead minnows, "no-effect" concentrations were between 0.52 and 1.0 $mg \cdot L^{-1}$ for alachlor, 14.5 and 48.5 $\mu g \cdot L^{-1}$ for dinoseb, 33.4 and 70.8 $\mu g \cdot L^{-1}$ for diuron, and 0.4 and 0.6 $\mu g \cdot L^{-1}$ for propanil. Growth was significantly reduced at the lowest bromacil exposure of 1.0 $mg \cdot L^{-1}$, but a "no effect" range was not determined.

All five herbicides had weak potentials for concentration from the water into fish tissue. Bioconcentration factors for the parent compounds in fathead minnows were 6.0, <3.2, 1.4, 2.0, and 1.6 for alachlor, bromacil, dinoseb, diuron, and propanil, respectively.

Rainbow trout injected with the radiolabeled herbicides eliminated over 75% of the radioactivity within 24 hr. The radioactivity was eliminated both as parent compound and as various metabolites.

One metabolite present in the bile of rainbow trout exposed to propanil was characterized by gas chromatography / mass spectrometry (GC/MS) as either

3',4'-dichloro-2-hydroxypropionanilide or 3',4'-dichloro-3-hydroxypropionanilide. Mass spectral data confirmed that 3,4-dichloroaniline had been eliminated into the water by trout injected with radiolabeled diuron.

All of the herbicides exhibited time-dependent binding to macromolecules. The binding was not dependent on NADPH, was not altered by the addition of glutathione, and was not altered by the substitution of heat-inactivated microsomes for active microsomes. Thus, the binding appeared to be due to the affinity of the parent herbicides for protein and other macromolecules and was not dependent upon mixed-function oxidase activity.

In unsterilized Lake Superior water held at room temperature and normal laboratory lighting conditions (fluorescent lights), propanil concentrations declined curvilinearly, with an estimated half-life of 65 days. The principal breakdown product in the propanil solution was identified by GC/MS as 3,4-dichloroaniline. Dinoseb concentrations declined by 21% in 40 days. Bromacil and diuron concentrations remained stable over 40 days. Alachlor was not tested for stability in solution.

The technical grade propanil used in this study also contained 0.67 $mg \cdot g^{-1}$ of 3,3',4,4'-tetrachloroazobenzene (TCAB) as a contaminant. No 3,3',4,4'-tetrachloroazoxybenzene (TCAOB) was detected in the propanil. Neither TCAB nor TCAOB were detected in the technical grade diuron used.

SECTION III

RECOMMENDATIONS

The effects of technical grade propanil on a variety of aquatic organisms should be further investigated in the laboratory and in the field under normal use patterns. Propanil residues in waters where the fauna may be impacted should be regularly monitored in conjunction with studies on possible biological effects.

The effects of trace amounts of 3,3',4,4'-tetrachloroazobenzene, a contaminant of technical propanil and diuron, on aquatic animals should be determined. Acute and chronic toxicity data as well as more field residue data are needed.

More surface water residue studies are needed in areas where diuron and dinoseb are used to determine if further laboratory and/or field investigations are warranted.

The relatively high concentrations of alachlor and bromacil necessary to produce adverse effects in fish would not likely be encountered in aquatic ecosystems. It is recommended that further studies on the direct effects of these two herbicides on aquatic animals be preceded by studies on other chemicals for which the potential hazard to aquatic life appears to be greater.

SECTION IV

METHODS

Water Supply and Environmental Control

Water for toxicity and bioconcentration tests was from Lake Superior. Several chemical parameters (dissolved oxygen, pH, hardness, acidity, and alkalinity) were monitored throughout the tests by standard analytical methods (American Public Health Association, 1975). Water was not filtered or sterilized. A portion of the water was heated (>30°C) before being distributed to the test systems.

Temperature control was maintained by proportional mixing of heated and unheated lake water in constant head reservoirs. Electronic sensors monitored the temperatures in the reservoirs and added heated water when necessary to maintain a desired water temperature of 25°C. Lighting for the acute and embryo-larval toxicity tests was supplied by two 40-watt fluorescent bulbs centered above the exposure chambers.

Test Organisms

Fathead minnow brood fish were received from stock maintained by the Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency. Brood fish were maintained at 25°C, and were fed twice daily a diet of frozen adult brine shrimp.

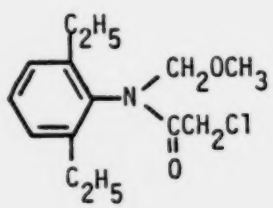
Asbestos pipe (12.5 cm O.D.) cut in half, longitudinally, was used as the spawning substrate. The spawning substrates (tiles) were checked daily for

egg deposition. Eggs were removed from the tiles the same day (<24 hr) that spawning occurred when used in early life-stage tests. For the culturing of fathead minnows to be used in acute toxicity or bioconcentration tests, tiles with "eyed up" eggs were placed in temperature controlled (25°C) hatching chambers to complete incubation. Once they had hatched (approximately 96 hrs after spawning at 25°C) fry were transferred to rearing aquaria where they were fed fresh, newly hatched brine shrimp nauplii 3 times daily.

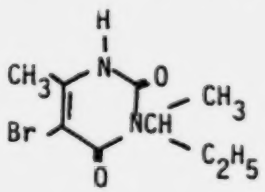
Rainbow trout ranging in size from 75 to 225 g were used for *in vivo* metabolism studies. Larger fish were used for *in vitro* metabolism studies. Trout were received from the Environmental Research Laboratory-Duluth, where they had been reared at 12°C.

Test Chemicals

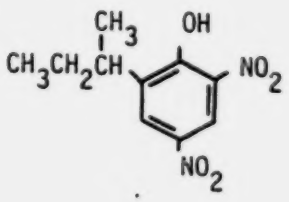
Alachlor. Non-radioactive alachlor or Lasso® was supplied by Monsanto Chemical Co. as technical grade material with a purity of 92.6% (lot no. MHF-183). ^{14}C-Labeled alachlor was uniformly ring-labeled compound with a specific activity of 1.9 $mCi \cdot mmol^{-1}$. Alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide] is an amide herbicide with the formula $C_{14}H_{20}ClNO_2$, a molecular weight of 269.9, and the following chemical structure (U.S. EPA, 1974; Agriculture Canada, 1973):



Bromacil. Non-radioactive bromacil was supplied by DuPont Chemical Co. as technical grade material with a purity of 95.0%. ^{14}C-Labeled bromacil was supplied by DuPont Chemical Co. from New England Nuclear (lot no. 896-232). It was radiolabeled at the number 2 carbon position, and had a specific activity of 6.25 $mCi \cdot mmol^{-1}$. Bromacil (5-bromo-3-sec-butyl-6-methyluracil) is a uracil derivative with the formula $C_9H_{13}BrN_2O_2$, a molecular weight of 261.1, and the following chemical structure (U.S. EPA, 1974; Agriculture Canada, 1973):

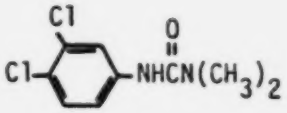


Dinoseb. Non-radioactive dinoseb (Premerge®) was supplied by Dow Chemical Co. as technical grade material with a purity of 98.0% (lot no. AGR 133942). ^{14}C-Labeled dinoseb was supplied by Dow Chemical Co. from Pathfinder Laboratories (lot no. 80612). It was uniformly ring-labeled with a specific activity of 7.80 $mCi \cdot mmol^{-1}$. Dinoseb (2-sec-butyl-4,6-dinitrophenol) is a phenolic herbicide with the formula $C_{10}H_{12}O_5N_2$, a molecular weight of 240.2, and the following chemical structure (U.S. EPA, 1974; Agriculture Canada, 1973).

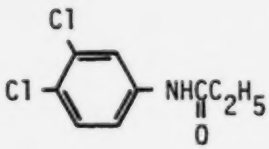


Diuron. Non-radioactive diuron (Karmex®) was supplied by DuPont Chemical Co. as technical grade material with a purity of 98.6%. ^{14}C-Labeled diuron was supplied by DuPont Chemical Co. as carbonyl ^{14}C-labeled material with a

specific activity of 0.98 $mCi \cdot mmol^{-1}$. ^{14}C-Labeled diruon in the form of uniformly ring-labeled compound was supplied by California Bionuclear Corporation (lot no. 780627AB) with a specific activity of 12.2 $mCi \cdot mmol^{-1}$. Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] is a phenylurea herbicide with the formula $C_9H_{10}Cl_2N_2O$, a molecular weight of 233.1, and the following chemical structure (U.S. EPA, 1974; Agriculture Canada, 1973):



Propanil. Non-radioactive propanil or STAM® was supplied by Rohm and Haas Chemical Co. as technical grade material with a purity of 85.9% (lot no. 8771). ^{14}C-Labeled propanil was also supplied by Rohm and Haas from New England Nuclear (lot no. 862-186). It was uniformly ring-labeled, and had a specific activity of 10.42 $mCi \cdot mmol^{-1}$. Propanil (3',4'-dichloropropionanilide) is an amide herbicide with the formula $C_9H_9NOCl_2$, a molecular weight of 218.1, and the following chemical structure (U.S. EPA, 1974; Agriculture Canada, 1973):



Toxicity Tests

Acute Toxicity Tests. Acute tests were run in 17.5 L glass aquaria (20 cm x 35 cm x 25 cm) containing 6 L of water. Water was delivered through proportional diluter systems (Mount and Brungs, 1967) with five toxicant concentrations and a control. Each toxicant concentration and control was run in

duplicate. The diluters delivered 0.5 L to each test tank 4 to 8 times per hour depending upon the toxicant used.

Twenty 30-day-old fathead minnows were placed into each aquarium. Observations for mortality were made several times during the first 24 hours and twice daily for the remaining 7 days of the test. Fish were not fed during the test. Fish were considered dead, and removed from the aquaria if opercular movements had ceased.

Water temperatures were measured two or more times weekly. All tests were run at a nominal water temperature of 25°C.

Toxicant stock solutions of alachlor, dinoseb, diuron, and propanil were generated from sand columns, adaptations of a procedure described by Veith and Comstock (1975). A near-saturation solution of these four herbicides in Lake Superior water was maintained by circulating the stock solution through a sand column containing the toxicant.

The bromacil stock solution was prepared differently due to the high concentrations required to kill fathead minnows. Bromacil was dissolved at near-saturation levels in a large reservoir (230 L) of Lake Superior water. This solution was continuously pumped to a head reservoir (17 L), from which it was dispensed to the mixing cell of the proportional diluter.

Early Life-Stage Tests. Early life-stage tests were run using the same basic diluter and exposure system as in the acute tests. The system was modified to permit incubation of eggs in oscillating 130 mL specimen bottles with 200 μm mesh nylon screen bottoms. The bottles were oscillated by a rocker arm assembly at a rate of about 6 times per minute, causing the eggs to move a vertical distance of about 5 cm. Modifications were also made within the aquaria to reduce the area and confine the fish. A glass and stainless steel

mesh (200 μm) barrier reduced the volume of water to 4.5 L. The bottom color of the aquaria was changed from black to white, permitting easier observation of fry.

Fathead minnow eggs <24 hours old were placed into the incubation jars (50 eggs per jar; 2 jars per treatment replicate). Dead eggs were removed daily, and upon completion of hatch the survivors were counted and 15 fry from each incubation jar were released into the aquarium (30 per aquarium). Feeding was begun the day after hatching and continued to the end of the test. Finely granulated dry fish food (Tetramin®) and newly hatched brine shrimp were fed for the first 30 to 45 days after hatching and supplements of frozen adult brine shrimp were added to the diet for the last 15 to 30 days of the test. Equal volumes of food were provided to each aquarium.

Several parameters were measured in the early life-stage tests. These included egg hatchability, occurrence of abnormal and dead fry at time of transfer from egg cups into the aquaria, fry survival at end of exposure period, and wet weight and length at end of exposure period. Observations were also made on behavior and development of the fry throughout the exposures.

Bioconcentration Tests

Bioconcentration tests were run in a modified diluter system using fluid metering pumps to deliver the test compounds (^{14}C-labeled) to each 27-L treatment aquarium. Water (1 L at 25°C) was delivered 4 times per hour to each treatment and control aquarium where it was mixed with the test compound before going into the aquarium. Each test solution was a mixture of technical grade and radiolabeled compound dissolved in methanol. Two exposure concentrations of herbicide were used which differed by approximately one order of magnitude. All aquaria including the controls received 100 $mg \cdot L^{-1}$ methanol.

One hundred fathead minnows (30 days old) were placed into each aquarium and exposed to the test compounds. Five fish were removed and individually analyzed on days 1, 2, 4, 7, 10, 14, 17, etc. until an equilibrium was achieved in total ^{14}C between the exposure water and the fish tissue. When equilibrium was reached, the remaining fish were transferred to 27-L aquaria receiving only Lake Superior water (no herbicide or methanol), with a turnover rate of 4 L·hr^{-1}. Elimination rates were determined by individually analyzing samples of 5 fish on days 1, 2, 4, 7, 10, 14, 17, etc. until ^{14}C activity had been almost completely eliminated.

Measurements of Herbicide Concentrations in Water and Fish

Alachlor. For the acute and embryo-larval toxicity tests, alachlor was extracted from water with a hexane:acetone (90:10, v/v) mixture. A 10 mL water sample was extracted three times with 5 mL of solvent mixture. The extract was collected in a 100 mL volumetric flask for the acute test samples and in a 500 mL Kuderna-Danish evaporative concentrator for the embryo-larval test samples. Acute test samples were diluted with hexane to appropriate volumes for GLC analysis. Embryo-larval test samples were concentrated on a steam bath prior to analysis.

Gas-liquid chromatographic (GLC) analysis was performed on a Tracor MT 160 instrument equipped with a ^{63}Ni electron capture detector and a glass column (6' x 1/4") packed with 3% OV-101 on 100/120 mesh Gas-Chrom Q. Nitrogen was the carrier and detector purge gas with flow rates of 34 and 35 mL·min^{-1}, respectively. Operating temperatures for inlet, column and detector were 222°C, 236°C, and 263°C, respectively. The retention time of alachlor was 3.72 minutes. At an output attenuation of 8 X, a 0.60 ng injection gave a 19% full scale deflection. The linear working range was 0.20 to 0.60 ng.

Standard curves were prepared daily by injecting several volumes up to 6 µL of a 0.10 $ng \cdot \mu L^{-1}$ working standard. Samples were diluted or concentrated to yield responses within the linear range of the gas chromatograph, and concentrations determined from a standard curve regression equation. Based upon 20 spikes in Lake Superior water ranging in concentration from 0.2 to 3.0 $mg \cdot L^{-1}$, the mean alachlor recovery was 92.8 ± 9.1%. Water concentrations were corrected on the basis of this recovery.

For the alachlor bioconcentration study, ^{14}C-alachlor was extracted from water with a mixture of benzene and isobutanol (50:50, v/v). A 100 mL water sample was extracted with 25 mL benzene:isobutanol and a 5 mL aliquot of the solvent layer was added to 15 mL of scintillation fluid. The scintillation fluid consisted of Permafluor III,® (Packard Instrument Co.) Triton X-100,® and toluene (10:33:57, v/v). Samples were counted for a minimum of 5 minutes along with standards on a Packard Tricarb liquid scintillation counter.

Concentration-count relationships were determined for ^{14}C-alachlor by using 4 duplicated standards. Background counts and instrument quench curve readings were made with each series of runs on the scintillation counter. A computer program corrected counts from standards and samples for differences in instrument quench readings, and calculated µg quantities of ^{14}C parent compound equivalents in water based on a regression line fit (least squares) from parent compound standard curves. Sample concentrations were then calculated and expressed in $\mu g \cdot L^{-1}$ of water. Due to water solubility of the solvent mixture, the solvent volume was reduced from 25 mL to 17.87 mL (n=6). Concentrations were corrected for this reduction, and recovery of ^{14}C-alachlor from Lake Superior water averaged 102.6 ± 2.9%, (n=22).

^{14}C-Alachlor was measured as parent compound equivalents in whole fish at

various time intervals during the bioconcentration study. Whole fish were blotted dry, weighed, and oxidized in a Packard Tri-Carb Model B306 Sample Oxidizer. Weighed fish were placed in a combustible cone, 0.3 mL of Combustaid® (Packard Instrument Co.) solution was added, and the fish were oxidized. The liberated ^{14}C-labeled CO_2 was trapped with 6 mL of Carbosorb® (Packard Instrument Co.), then rinsed and diluted with 14 mL of Permafluor V® (Packard Instrument Co.). The solution was counted in a liquid scintillation counter, and the counts quantitatively equated to parent compound based upon an alachlor standard curve. Recovery of ^{14}C-alachlor from fish averaged 86.8 ± 5.8%, (n=15). Sample concentrations were then calculated and expressed in $\mu g \cdot g^{-1}$ of fish tissue.

Bromacil. For acute and embryo-larval tests, bromacil water concentrations were measured on a Beckman UV-visible double-beam spectrophotometer at a wavelength of 280 nm. Bromacil standard solutions in methanol ranging in concentration from 0.8 to 10 $mg \cdot L^{-1}$ were used to prepare standard curves on each sampling day. Exposure chamber water samples were either measured directly, as in some of the lower concentrations in the embryo-larval test, or diluted with distilled water to fall within the absorbance range of the standards. The absorbances of the control chambers were subtracted as correction factors in calculating exposure tank concentrations of bromacil. The minimum detection limit for bromacil was approximately 0.5 $mg \cdot L^{-1}$.

^{14}C-Bromacil water concentrations in the bioconcentration study were determined by methylene chloride extraction. Water samples of 100 mL were extracted with 25 mL of methylene chloride. The solvent was evaporated to dryness in a 60°C hot water bath under a hood. The residue was dissolved in 5.0 mL of toluene, 15.0 mL of scintillation fluid (Permafluor III®, Triton X-

100®, and toluene, 10:33:57, v/v) were added, and the sample counted on a scintillation counter as described for alachlor. Recovery of ^{14}C-bromacil from Lake Superior water was 87.5 ± 2.6%, (n=20).

^{14}C-Bromacil was measured as parent compound equivalents in whole fish at various time intervals during the bioconcentration study. The procedure was the same as described previously for ^{14}C-alachlor. The mean recovery of ^{14}C-bromacil was 88.1 ± 4.2%, (n=10).

Dinoseb. In the acute toxicity test where all dinoseb concentrations exceeded 0.10 mg·L^{-1}, dinoseb was extracted from water by placing a 10.0 mL sample into a 60 mL separatory funnel. Concentrated sulfuric acid (2-4 drops) was added to lower the pH below 2.0, followed by the addition of 0.20 mL of saturated NaCl solution. Dinoseb was then triple-extracted with 10 mL of benzene. After each extraction, the benzene layer was drained through a 60° funnel plugged with glass wool (acetone washed) and filled with 20 g of anhydrous sodium sulfate. The separatory funnel was rinsed with 10 mL of benzene which was also passed through the sodium sulfate. The extracts were collected in a 25 mL volumetric flask and diluted to volume, prior to methylation.

In the embryo-larval toxicity test, where all dinoseb concentrations were less than 0.10 mg·L^{-1}, 500 mL water samples were collected. To each sample, approximately 3 mL of concentrated sulfuric acid and 10 mL of saturated NaCl solution were added. A triple-extraction with 50, 25, and 25 mL of benzene was made, with the extracts collected in a 500 mL Kuderna-Danish evaporative concentrator. The sample was concentrated on a steam bath to approximately 2 mL, and further reduced to 1 mL under a stream of nitrogen gas. The concentrated extract was then methylated.

Diazomethane was prepared daily with each set of samples according to the procedure in "Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples," (U.S. EPA, 1977). Under a ventilated hood, small increments of N-methyl-N'-nitro-N-nitrosoguanidine (Aldrich Chemical Co.) crystals were added to a test tube containing 5.0 mL of 20% NaOH and 3 mL of hexane, until the solution was saturated with diazomethane. The reagent was used immediately after gas bubbles had ceased evolving.

Dinoseb sample extracts from both the acute and embryo-larval tests were methylated by adding approximately 12 drops of the diazomethane-saturated hexane layer to 1.0 mL of sample extract. The reaction flask was swirled and allowed to stand for at least 15 minutes. The methylated extract was then diluted to an appropriate volume for GLC analysis.

GLC analysis of dinoseb was performed on Tracor MT 160 and Tracor 550 instruments, both equipped with ^{63}Ni electron capture detectors. The Tracor MT 160 glass column (6' x 1/4" i.d.) was packed with 3% OV-101 on 100/120 mesh Gas-Chrom Q. Inlet, column, and detector temperatures were 230, 205, and 260°C, respectively. Nitrogen was the carrier and detector purge gas at flow rates of 45 and 36 $mL \cdot min^{-1}$, respectively. With an attenuation of 2 X, 0.05 ng of dinoseb produced a 25% full-scale deflection. The Tracor 550 glass column was 6' x 1/4" i.d. packed with 3% OV-101 on 80/100 mesh Chromosorb W. Inlet, column, and detector temperatures were 223, 196, and 259°C, respectively. Nitrogen was the carrier gas with a flow rate of 65 $mL \cdot min^{-1}$ and the detector purge gas at a flow rate of 49 $mL \cdot min^{-1}$. With an attenuation of 4 X, 0.05 ng produced a 28% full-scale deflection.

Dinoseb standard solutions were prepared from a 1.0 $mg \cdot L^{-1}$ stock solution

in acetone. A 1.0 $\mu g \cdot mL^{-1}$ intermediate standard was prepared by dilution of the stock with benzene. Methylation was performed on 1.0 mL of this standard, which was then diluted with hexane to give a working standard of 0.01 $\mu g \cdot mL^{-1}$. Standard curves were prepared daily by injecting from 1 to 5 μL, and plotting nanogram quantity versus peak height. The detection limit for dinoseb using a 500 mL water sample was 0.005 $\mu g \cdot L^{-1}$.

Dinoseb recovery from water was determined by spiking Lake Superior water over a range of concentrations from 0.50 $\mu g \cdot L^{-1}$ to 1.00 $mg \cdot L^{-1}$. The mean recovery was 96.4 + 8.7%, (n=22).

^{14}C-Dinoseb was extracted from water in the bioconcentration study with benzene:isobutanol, (50:50 v/v). A 100 mL sample was acidified with 2 drops of concentrated H_2SO_4 and extracted with 25 mL benzene:isobutanol. A 5 mL aliquot of the solvent layer was added to 15 mL of scintillation fluid (same as described previously) and counted as described for alachlor. After correction for solvent volume reduction, recovery of ^{14}C-dinoseb from Lake Superior water was 102.5 ± 3.2%, (n=20).

^{14}C-Dinoseb concentrations in whole fish were measured as described for ^{14}C-alachlor, using a sample oxidizer, and were expressed as parent compound equivalents. The mean recovery from fish tissue was 78.2 ± 9.6% (n=20).

Diuron. Diuron water concentrations in the acute toxicity test were measured with a Beckman double-beam UV-visible spectrophotometer at a wavelength of 250 nm. Diuron standards were prepared in methanol and ranged in concentration from 0.6 to 4.0 $mg \cdot L^{-1}$. Standard curves were prepared daily with each set of analyses, and exposure chamber concentrations were determined from the standard curve regression equation. Chamber water samples were diluted as necessary with distilled water to give absorbance readings within

the standard curve concentration limits. The absorbance readings of control tanks were subtracted from samples as correction factors in calculating tank concentrations. The minimum detection limit was 0.1 $mg \cdot L^{-1}$.

Diuron water concentrations in the embryo-larval test were below the UV-visible spectrophotometer sensitivity, ranging from means of 2.0 to 61.8 $\mu g \cdot L^{-1}$. For this test, diuron water concentrations were determined by extraction with methylene chloride and analysis by high pressure liquid chromatography (HPLC), based upon the procedure of Farrington et al. (1977).

A 500 mL water sample was triple extracted with methylene chloride (50, 25, 25 mL), and the solvent collected in a 500 mL Kuderna-Danish evaporative concentrator. The methylene chloride was reduced to a volume of approximately 2 mL on a steam bath, and further evaporated to dryness under a stream of nitrogen gas. The residue was redissolved with 5.0 mL of methanol (spectroscopy grade), and diluted as appropriate with methanol for HPLC analysis.

HPLC analysis was performed on a Waters Associates Model 6000A instrument equipped with a Model 440 UV absorbance detector with a fixed wavelength of 254 nm. A stainless steel column (300 x 2 mm i.d.) was used, packed with Porasil-18 and operated at ambient temperature. The mobile phase was 60% methanol and 40% water (deionized and degassed) with a flow rate of 2 $mL \cdot min^{-1}$ and operated at a pressure of approximately 3800-4000 psi. A WISP (Waters Intelligent Sample Processor) automatic sample injection system was used, with a standard injection volume of 20 µL for all samples and standards.

Diuron standards in methanol ranging in concentrations from 0.125 $ng \cdot \mu L^{-1}$ to 1.00 $ng \cdot \mu L^{-1}$ were run daily with each set of analyses. Sample extracts were adjusted to fall within this range of concentrations, and sample concentrations were calculated from the standard curve regression equation. The

retention time of diuron was 3.83 minutes. At an attenuation of 1X, a 20 ng injection produced a 37% full-scale recorder deflection. The linear range of the detector for diuron was 2.5 to 40 ng. At concentrations of spiked samples ranging from 5 $\mu g \cdot L^{-1}$ to 50 $\mu g \cdot L^{-1}$, the mean recovery from Lake Superior water was 88.9 ± 6.0%, (n=19).

Propanil. For the acute toxicity test, where mean propanil concentrations ranged from 1.9 to 15.7 $mg \cdot L^{-1}$, 10 mL water samples were collected and triple-extracted with hexane (10, 5, and 5 mL). The extracts were diluted to appropriate volumes for GLC analysis.

In the propanil embryo-larval test, where mean propanil concentrations ranged between 0.4 and 3.8 $\mu g \cdot L^{-1}$, 100 mL water samples were collected and triple-extracted with 25 mL portions each of hexane. The combined extracts were collected in a Kuderna-Danish concentrator, and concentrated on a steam bath to appropriate volumes for GLC analysis.

Two gas chromatographs were used for analysis of propanil concentrations. A Tracor 220 instrument equipped with a ^{63}Ni electron capture detector was used for samples between 1.0 and 20.0 $mg \cdot L^{-1}$. The glass column (6' x 1/4" i.d.) was packed with 3% OV-101 on 80/100 mesh Chromosorb W-HP. Argon-methane (95:5) was the carrier and detector purge gas with a flow rate of 50 $mL \cdot min^{-1}$ through both the column and detector. An output attenuation of 4X was used. Operating temperatures for inlet, column, and detector were 205°C, 193°C, and 300°C, respectively. The retention time of propanil was 3.05 minutes, and an injection of 0.80 ng gave a 17% full scale recorder deflection. The linear working range was 0.4 to 1.0 ng.

A Tracor 550 instrument equipped with a ^{63}Ni electron capture detector was used for samples between 0.1 and 10.0 $\mu g \cdot L^{-1}$. The glass column (6' x

1/4" i.d.) was packed with 3% OV-101 on 80/100 mesh Chromosorb W. Nitrogen was the carrier and detector purge gas with flow rates of 60 and 30 $mL \cdot min^{-1}$, respectively. An output attenuation of 2x was used. Operating temperatures for inlet, column, and detector were 218°C, 196°C, and 240°C, respectively. The retention time of propanil was 3.22 minutes, and an injection of 0.20 ng gave a 35% full scale recorder deflection.

A stock solution of 1.0 $mg \cdot mL^{-1}$ propanil (95% purity) was prepared in acetone. Standard solutions of propanil were prepared by making further dilutions with hexane. The working standards used for GLC analyses contained 0.01 $ng \cdot \mu L^{-1}$ and 0.10 $ng \cdot \mu L^{-1}$ propanil. Standard curves were prepared daily with each set of analyses by plotting peak height versus nanogram quantity of standards (0.05, 0.10 and 0.20 ng), and sample concentrations determined from the standard curve regression equation.

The recovery of propanil from Lake Superior water was determined by spiking the water over a range of concentrations between 0.20 $mg \cdot L^{-1}$ to 200 $mg \cdot L^{-1}$. Based upon 24 determinations, the mean recovery was 98.5 ± 3.3%.

^{14}C-Propanil was extracted from water in the bioconcentration study with toluene. A 500 mL water sample was extracted with 25.0 mL of toluene. A 5.0 aliquot of the solvent layer was placed into a scintillation vial, and 15.0 mL of scintillation fluid (same composition as before) were added. Samples were then counted and concentrations determined, as described for alachlor. The mean recovery from Lake Superior water was 92.8 ± 1.9% (n=32).

^{14}C-Propanil concentrations in whole fish were measured as described for ^{14}C-alachlor, using a sample oxidizer, and were expressed as parent compound equivalents. The mean recovery from fish spiked with ^{14}C-propanil was 90.9 ± 4.5% (n=20).

Metabolite Characterization

Rainbow Trout in vivo Experiments. Rainbow trout (Salmo gairdneri) from stock maintained at ERL-Duluth were used for initial in vivo herbicide metabolism experiments. Individual fish weighing 100-150 g were tranquilized with tricaine methane sulfonate (MS 222) at a concentration of 50 $mg \cdot L^{-1}$, and transferred to a small aquarium in which the water temperature was maintained at $10^{o}C$. The aquarium was shaded on the sides and top, and each fish was allowed to remain undisturbed for 24 hours. On the following day, the fish were lightly anaesthetized with MS 222 (50 $mg \cdot L^{-1}$), and injected with 1 µCi of ^{14}C-labeled herbicide. After an additional 24 hours, the trout were anaesthetized with MS 222 (100 $mg \cdot L^{-1}$), blood taken from the caudal artery, bile removed from the gall bladder with a 1 cc syringe, and the liver excised. The liver, bile, blood, and aquarium water were analyzed for metabolites.

Following the initial in vivo experiments, rainbow trout weighing 100-150 g were exposed for 4-5 days to unlabeled herbicide dissolved in 10-12 L of Lake Superior water at concentrations below acutely lethal levels (e.g. propanil at 1.0 $mg \cdot L^{-1}$). Water temperature was maintained at $10^{o}C$, and the water changed after the first 24 hrs to remove fecal material. The fish were not fed during the exposure period. One day prior to sacrifice, individual fish were injected with 1.0 µCi of ^{14}C-labeled herbicide. The fish were killed, the liver excised, and the gall bladder carefully separated from the liver. The gall bladder, bile, and liver were analyzed for metabolites.

Metabolite Recovery from Water. Aquarium water containing radiolabeled alachlor, bromacil, diuron, or propanil metabolites was passed through a column (2.5 x 30 cm) of Amberlite XAD-2 resin. The column was washed with

1-2 bed volumes of distilled water, then eluted with 900 mL of methanol. Aliquots of the water prior to XAD-2 treatment and of the methanol eluate were counted in a liquid scintillation counter to determine total radioactivity recovered from the column. The methanol eluate was evaporated to dryness in a stream of air prior to TLC. Dinoseb did not absorb well to the XAD-2 resin; consequently, dinoseb aquarium water was acidified with HCl to a pH of 2 and passed through a column of Sephadex LH20. The column was eluted with methanol, and the eluate evaporated to dryness.

Metabolite Recovery from Liver. Fish liver was homogenized with 20-25 volumes of cold acetone in a Waring blender, followed by centrifugation for 5 min. at 900 X g to remove insoluble solids. The acetone extract was dried with sodium sulfate and then evaporated in a stream of air. An aliquot of the acetone extract was counted in a scintillation counter and the solid residue processed in a Packard Sample Oxidizer to determine unextracted radioactivity.

Metabolite Recovery from Bile. Bile was diluted 5:1 with water, acidified with HCl to a concentration of 0.1 M, and extracted three times with 10 mL of ethyl ether. The ether extract was evaporated to dryness in a steam of nitrogen, and the residue redissolved in acetone prior to TLC. After TLC, the chromatograms were examined under ultra-violet light for absorbing spots or bands, and radioactive areas located with a Packard radiochromatogram scanner. Areas which were both radioactive and ultra-violet absorbing were scraped to remove the silica gel, and the silica gel eluted with methanol. The eluted material was then analyzed by gas chromatography/mass spectrometry (GC/MS).

Metabolite Recovery from Blood. Whole blood was centrifuged for 20 min at 900 X g. The plasma was diluted with 3.5 volumes of acetone followed by centrifugation at 900 X g for 5 min. to remove precipitated protein. The extract was loaded on an XAD-2 column (1 x 25 cm), and processed as described above for bile. Aliquots of the extract were counted by liquid scintillation techniques, and the precipitated solids burned in a sample oxidizer for radioactivity determinations.

The packed cells were washed twice with isotonic saline followed by 2 mL of acetone. The suspension was centrifuged for 5 min at 900 X g, and the extract loaded on a 1 x 25 cm XAD-2 column as described above. Radioactivity in the extract and residual solid was determined as described for plasma.

Thin-Layer Chromatography. Chromatography was done on commercially available silica gel coated Mylar® sheets (Eastman Co.) with a fluorescent indicator. Samples to be analyzed by mass spectroscopy were chromatographed on glass plates coated with silica gel containing a fluorescent indicator. Chromatogram development was in an Eastman "Chromagram" development apparatus.

The following TLC solvent systems were used for alachlor and its metabolites - hexane: ethyl acetate (97:3, v/v); for bromacil and its metabolites - toluene:ethyl acetate (80:20, v/v); and for dinoseb plus metabolites - toluene:n-butanol (60:40, v/v). Propanil extracts were initially developed with 85% toluene and 15% methanol. The chromatogram was developed a second time with 65% toluene and 35% methanol with the solvent allowed to migrate only 40% of the original distance to prevent displacement of the previously separated less polar components. The chromatogram was developed a third time with 35% toluene and 65% methanol to 20% of the original migration distance. By utilizing multiple development, all metabolites were forced to migrate

from the origin.

Gas Chromatographic-Mass Spectrometric (GC/MS) Characterization of Metabolites

Analyses were performed on extracts of exposure water or fish bile with a Finnigan Model 4023 A at the Environmental Research Laboratory-Duluth (U.S. Environmental Protection Agency). A Finnigan INCOS 2300 data system was used on line to acquire and process mass spectral data.

A glass GC column (6' x 1/8" I.D.) was packed with 3% OV-1 (methyl silicone) on 60/80 mesh Gas-Chrom Q. The following GC conditions were typically employed: helium carrier gas flow rate, 20 $mL \cdot min^{-1}$; injector temperature, 250°C; separator oven temperature, 280°C; transfer line temperature, 280°, and column oven temperature programmed from 100°C to 225°C at $4^{o}C \cdot min^{-1}$.

The mass spectrometer was scanned 50 to 500 at 2.05/decade in electron impact mode (70 ev). The instrument was tuned to provide a 442/198 amu ratio of 0.7 - 0.9 for the spectrum of Ultramark 443 (decafluorotriphenylphospine, PCR Research Chemicals, Inc.). Calibration was accomplished with FC43 (perfluorinated tributyl amine).

Rainbow Trout Liver in Vitro Experiments. A freshly excised rainbow trout liver was rinsed with 1.15% KCl, and homogenized with two volumes of 1.15% KCl in a Potter-Elvehjem homogenizer. The homogenate was centrifuged for 20 minutes at 10,000 X g at 4°C, and the supernatant liquid used as the enzyme source. Incubation mixtures contained 0.1 M tris-acetate, pH 7.5, 0.005 M $MgCl_2$, 0.2 mM NADPH, 0.05 µCi of ^{14}C-labeled herbicide, and 0.2 mL of enzyme preparation in a total volume of 1.0 mL. Incubation was for 2

hours at 25°C. After the incubation period, the mixture was acidified with HCl to 0.1 M and extracted three times with 10 mL ethyl ether. The ether extract was evaporated to dryness in a stream of nitrogen, the residue redissolved in acetone, and the extract analyzed by thin-layer chromatography (TLC) and radiochromatogram scanning.

In Vitro Binding of Herbicides to Macromolecules. Fresh rainbow trout livers were homogenized and centrifuged as above, with the supernate used as the source for metabolizing enzymes. Incubation mixtures were prepared as above, with incubation initiated by the addition of the enzyme source.

At various times, 0.1 mL aliquots were removed from the incubation mixture and pipetted onto 2.0 x 2.5 cm pieces of blotting paper. After the paper absorbed the aliquot of incubation mixture, it was immersed in 70% ethanol to precipitate protein and other macromolecules. The paper was sequentially washed in 100% ethanol containing 5 $mg \cdot L^{-1}$ of unlabeled herbicide, acetone, and finally acetonitrile. The paper was then air dried, burned in a Packard Sample Oxidizer, and counted in the liquid scintillation counter to determine radioactivity.

Fathead Minnow Metabolite Studies. Extra fish from each of the fathead minnow bioconcentration tests (excluding bromacil) were frozen at the end of the uptake phase of each test. Pooled whole fish samples (1.5 - 6.0 g) were homogenized with 6.5 mL of water in a Potter-Elvehjem homogenizer, and a 50 µL aliquot of the homogenate was analyzed for total ^{14}C radioactivity. The homogenate was acidified with 0.4 mL of concentrated HCl and extracted three times with 10 mL portions of ethyl ether. For the herbicides studied in this manner (alachlor, dinoseb, diuron, and propanil), ether extraction failed to

remove any radioactivity from the aqueous phase. The aqueous layer was centrifuged and the pellet oxidized in a Packard Model 306 Sample Oxidizer and counted in a liquid scintillation counter to determine particulate-bound ^{14}C. The supernatant was lyophilized, and the residue dissolved in a small volume of acetone. Acetone solutions of the water soluble fractions were applied on silica gel thin-layer strips, and co-chromatographed with appropriate standards of parent herbicides. A standard of the propanil metabolite, 3,4-dichloroaniline, was also co-chromatographed with the extract of the propanil-exposed fish. Thin-layer strips were developed with the following solvents (v/v): alachlor - 99:1 toluene:methanol; dinoseb and diuron - 90:10 toluene:methanol; and propanil - 92:8 toluene:methanol.

Developed thin-layer strips were first analyzed for ^{14}C activity on a radiochromatogram scanner. Radioactive bands were scraped from the silica gel strips, and compounds eluted from the silica gel with water followed by methanol in the case of the propanil-exposed fish extracts, and methanol alone for extracts of fish exposed to alachlor, dinoseb, and diuron. Eluates were added to scintillation fluid and counted to determine relative contributions of parent herbicides and metabolic products toward total ^{14}C in the water-soluble fractions.

Herbicide Stability in Solution

Solutions of bromacil, diuron, dinoseb, and propanil at concentrations approximating the 96 hr LC_{50} concentrations for fathead minnows were prepared using Lake Superior water. Bromacil, diuron, and dinoseb solutions were held in 1 L stoppered clear-glass bottles in duplicate, and were allowed to stand in a light (12 hr fluorescent light and 12 hr dark) and temperature

(25°C) controlled room for 40 days. Samples were taken from each bottle at various time intervals. Propanil stability was studied using 500 mL stock solutions in duplicate held under the same light conditions as the others but at 20°C and sampled over 194 days. Analytical techniques were the same as for the acute toxicity tests.

GC/MS Analysis of Technical Grade Propanil and Diuron

Technical grade propanil and diuron samples were analyzed for the presence and amount of 3,3',4,4'-tetrachloroazobenzene (TCAB) and 3,3'4,4'-tetrachloroazoxybenzene (TCAOB) by GC/MS. A Finnigan 4000 GC/MS with multiple ion detection (MID) was used, with a 15 m x 0.2 mm I.D. SE-30 fused silica capillary column operated in the splitless injection mode. The following GC/MS operating conditions were employed: He carrier gas at a linear velocity of 47 $cm \cdot min^{-1}$, injector temperature of 250°C, transfer temperature of 250°C, oven temperature programmed from 60-180°C at an initial hold of 0.5 min. and an increase of $10°C \cdot min^{-1}$, and then increased at a rate of $5°C \cdot min^{-1}$ between 180-250°C, with a 10 min. hold at 250°C; 70 eV electron impact, 0.35 MA emission current, 1750 V EM voltage, and 10^{-8} sensitivity. Samples were quantitated using p-diiodobenzene as an internal standard. The following ions were monitored for 0.210 sec during each 1.00 second MID scan period: TCAB and TCAOB, m/z 145 and 147; DIB, m/z 330.

SECTION V

RESULTS

Toxicity Tests

Acute Flow-Through Toxicity Tests. Chemical characteristics of the test water during the acute exposures are presented in Appendix A. Herbicide concentrations in the exposure chambers were corrected for recoveries of the test compounds from water.

Alachlor was tested at six duplicated control and exposure concentrations (means of 1.70, 3.19, 5.04, 8.56, and 14.8 $mg \cdot L^{-1}$). The mean water temperature was 24.7°C with a range from 23.6 to 25.8°C. LC_{50} values for the replicates combined at 96 and 192 hrs were 5.0 and 3.0 $mg \cdot L^{-1}$, respectively (Table 1).

Bromacil was tested at six duplicated control and exposure concentrations (means of 108, 127, 146, 172, and 207 $mg \cdot L^{-1}$). The mean water temperature was 25.0°C with a range from 24.1 to 25.9°C. LC_{50} values for the replicates combined at 96 and 168 hrs of exposure were 182 and 167 $mg \cdot L^{-1}$, respectively (Table 1).

Dinoseb was tested at six duplicated control and exposure concentrations (means of 0.24, 0.34, 0.50, 0.69, and 1.00 $mg \cdot L^{-1}$). The mean water temperature was 25.0°C and ranged from 24.0 to 25.8°C. LC_{50} values for the replicates combined at 96 and 192 hrs of exposure were 0.7 and 0.5 $mg \cdot L^{-1}$, respectively (Table 1).

TABLE 1. LC_{50} VALUES AND 95% CONFIDENCE INTERVALS FOR ACUTE TOXICITY TESTS WITH REPLICATES COMBINED (All Values Expressed in $mg \cdot L^{-1}$)

Compound	24 Hr LC_{50}	48 Hr LC_{50}	96 Hr LC_{50}	192 Hr LC_{50}
Alachlor	9.9 (9.3-10.6)	6.6 (6.3-7.0)	5.0 (4.5-5.6)	3.0 (2.6-3.5)
Bromacil	185 (179-191)	183 (177-189)	182 (177-188)	167 (162-174)*
Dinoseb	0.8 (0.8-0.9)	0.7 (0.6-0.8)	0.7 (0.6-0.7)	0.5 (0.4-0.5)
Diuron	23.3 (21.0-25.9)	19.9 (19.5-20.4)	14.2 (13.4-15.0)	7.7 (6.0-9.9)
Propanil	11.5 (10.8-12.2)	10.2 (9.6-10.8)	8.6 (7.7-9.5)	3.4 (2.4-4.8)

* Values are for 168 hr LC_{50}.

Diuron was tested at six duplicated control and exposure concentrations (means of 5.54, 7.94, 11.14, 16.42, and 24.20 $mg \cdot L^{-1}$). The mean water temperature was 24.3°C with a range from 22.3 to 25.6°C. LC_{50} values for pooled replicates of diuron at 96 and 192 hrs were 14.2 and 7.7 $mg \cdot L^{-1}$, respectively (Table 1).

Propanil was tested at six duplicated control and exposure concentrations (means of 1.9, 4.0, 8.4, 11.1, and 15.7 $mg \cdot L^{-1}$). The mean water temperature was 25.3°C with a range from 24.1 to 26.6°C. LC_{50} values for the pooled replicates at 96 and 192 hrs were 8.6 and 3.4 $mg \cdot L^{-1}$, respectively (Table 1).

Acute mortality curves for fathead minnows exposed to the five test herbicides are presented in Appendix B (Figs. B 1-5). The 95% confidence intervals for the two replicates were generally overlapping.

Early Life-Stage Flow-Through Toxicity Tests. Early life-stage tests were conducted for all herbicides by incubating and hatching fathead minnow eggs in the exposure water, and continuously exposing the fry and juvenile fish to herbicide concentrations for 54 to 60 days. Herbicide concentrations were corrected for recoveries of the test compounds from water. Chemical characteristics of the test water during the early life-stage exposures are presented in Appendix A.

Alachlor

Fathead minnow eggs (95 to 111 total per incubation chamber) less than 24 hrs old were exposed to alachlor at six duplicated control and exposure concentrations (means of 0.06, 0.14, 0.26, 0.52, and 1.10 $mg \cdot L^{-1}$). The mean water temperature was 25.3°C with a range from 23.0 to 28.6°C. Hatching success for controls averaged 72.8%, and ranged from 68.3 to 76.4% for the five alachlor exposure concentrations (Table 2). Hatching success was not affected

TABLE 2. HATCHABILITY, ABNORMAL DEVELOPMENT, SURVIVAL AND GROWTH OF FATHEAD MINNOWS (Pimephales promelas) EXPOSED TO ALACHLOR FOR 64 DAYS

Parameter	Mean Alachlor Concentration ± s.d. ($mg \cdot L^{-1}$)					
	0.0 ± 0.0 (n=20)	0.06 ± 0.02 (n=37)	0.14 ± 0.04 (n=39)	0.26 ± 0.04 (n=40)	0.52 ± 0.08 (n=40)	1.10 ± 0.18 (n=40)
Mean percent hatch[a]	72.8	73.3	68.3	76.2	76.4	73.4
Mean percent abnormal and dead[b]	2.6	4.0	3.7	2.0	0.6	0.0
Mean number of survivors at 60 days post-hatch[c]	24.5	26.5	28.0	28.0	29.5	25.0
Mean wet weight at 60 days post-hatch (g)	0.482	0.432	0.419*	0.403	0.423	0.316**
Mean total length at 60 days post-hatch (mm)	37.1	36.2	35.6*	35.0	35.9	31.9**

[a] Live fry/total eggs after 6 days.

[b] Abnormal (deformed) + dead fry/total fry at time of transfer from egg cups (6 days after initial exposure of eggs).

[c] Based on 30 fry transferred to duplicate exposure chambers.

* Significantly different from controls ($p<0.05$).

**Significantly different from controls ($p<0.01$).

by alachlor exposure. The mean percent of fry that were dead or abnormal at the time of transfer from egg incubation chambers to the aquaria proper was 2.6% for controls, and ranged from 0 to 4.0% for the five alachlor exposures. None of the alachlor exposures resulted in a significant increase in abnormal newly hatched fry ($p>0.05$). Survival of transferred fry through 60 days of exposure averaged 24.5 individuals out of 30 for controls, and from 25.0 to 29.5 for the five alachlor exposure concentrations. None of the alachlor exposures resulted in a significant decrease in fry survival through 60 days ($p>0.05$).

Mean wet weight for controls at 60 days was 0.482 g, and ranged from 0.316 to 0.432 g for the five alachlor exposure levels (Table 2). Differences in amounts of light incident upon the alachlor exposure aquaria due to non-uniform room lighting were noticed late in the exposure. Relative light values were obtained with a photometer at the water surface of each aquarium and ranged from 15.6 to 36.3. Multiple linear regression analysis indicated a significant ($p<0.01$) interaction between concentration, light, and fish weight or length. Fish weights and lengths were adjusted for influence of light from the multiple linear regression equations. Adjusted weights and lengths were then analyzed by one-way analysis of variance in conjunction with Dunnett's test. Fish were significantly lighter ($p<0.05$) at the exposure concentrations of 0.14 $mg \cdot L^{-1}$ ($p<0.05$) and 1.10 mg·L ($p<0.01$). Mean fry length at 60 days was 37.1 mm for controls, and ranged from 31.9 to 36.2 mm for the five alachlor exposure levels. Fry length was significantly reduced at exposure concentrations of 0.14 ($p<0.05$), and 1.10 ($p<0.01$) $mg \cdot L^{-1}$. Based on the highly significant decreases in weight and length of fry at 60 days at the 1.10 $mg \cdot L^{-1}$ exposure level and no effects at the next two lower exposures, the "no effect"

concentration for fathead minnows exposed to alachlor was considered to fall between 0.52 and 1.0 $mg \cdot L^{-1}$.

Bromacil

Fathead minnow eggs (92-106 total per incubation chamber) less than 24 hrs old were exposed to bromacil at six duplicated control and exposure concentrations (means of 1.0, 1.9, 4.4, 12.0, and 29.0 $mg \cdot L^{-1}$). The mean water temperature was 24.7°C with a range from 22.9 to 27.2°C. Hatching success for controls averaged 66.0%, and ranged from 58.4 to 72.3% for the five exposure levels (Table 3). Hatching success was not significantly affected ($p>0.05$) by bromacil. The percent of fry at time of transfer that were dead or abnormal averaged 5.9 for controls and ranged from 6.9 to 11.5% for the five exposure levels. None of the bromacil test concentrations resulted in a significant increase ($p>0.05$) in abnormal (including dead) newly hatched fry. Percent survival of transferred fry (60 total) at 60 days post-hatch averaged 88.3% for controls and ranged from 88.3 to 98.3% for the five bromacil exposure concentrations. Survival at 60 days was not significantly affected ($p>0.05$) by bromacil at these concentrations.

Weight was significantly reduced ($p<0.05$) from the mean control wet weight of 0.479 g at all five bromacil exposures (Table 3); and with the exception of the second lowest exposure (1.9 $mg \cdot L^{-1}$), weight was significantly reduced at the 99% confidence level by the remaining bromacil exposures. Length was significantly reduced ($p<0.05$) from the mean control length of 29.8 mm at all bromacil concentrations with the exception of the second lowest exposure (1.9 $mg \cdot L^{-1}$). Length was significantly reduced at the 99% confidence level by the three highest bromacil exposures (4.4, 12.0, and 29.0 $mg \cdot L^{-1}$), but not by the two lowest exposures (1.0 and 1.9 $mg \cdot L^{-1}$).

TABLE 3. HATCHABILITY, ABNORMAL DEVELOPMENT, SURVIVAL AND GROWTH OF FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO BROMACIL FOR 64 DAYS

Parameter	Mean Bromacil Concentration ± s.d. ($mg \cdot L^{-1}$)					
	0.0 ± 0.0 (n=34)	1.0 ± 0.4 (n=36)	1.9 ± 0.2 (n=36)	4.4 ± 0.5 (n=36)	12.0 ± 1.0) (n=33)	29.0 ± 2.1 (n=36)
Mean percent hatch[a]	66.0	68.8	58.4	58.5	60.4	72.3
Mean percent abnormal and dead[b]	5.9	6.9	7.4	8.3	8.4	11.5
Mean number of survivors at 60 days post-hatch[c]	26.5	28.5	29.5	29.5	27.5	26.5
Mean wet weight at 60 days post-hatch (g)	0.479	0.410**	0.420*	0.381**	0.374**	0.326**
Mean standard length at 60 days post-hatch (mm)	29.8	28.4*	28.7	27.9**	27.7**	26.4**

[a] Live fry/total eggs after five days.

[b] Abnormal (deformed) + dead fry/total fry at time of transfer from egg cups (5 days after initial exposure of eggs).

[c] Based on 30 fry transferred to duplicate exposure chambers.

* Significantly different from controls ($p<0.05$).

**Significantly different from controls ($p<0.01$).

A "no-effect" concentration for bromacil was not determined from the test. The fish showed an adverse response to bromacil at all tested concentrations using the parameter of weight at 60 days post-hatch. Only one exposure concentration (1.9 $mg \cdot L^{-1}$) did not show an adverse response using the parameter of length at 60 days post-hatch. It can only be concluded from this test that the "no effect" concentration for fathead minnows exposed to bromacil as eggs and for 60 days post-hatch is less than 1.0 $mg \cdot L^{-1}$.

Dinoseb

Fathead minnow eggs (96 to 109 total per incubation chamber) less than 24 hrs old were exposed to dinoseb at six duplicated control and exposure concentrations (means of 0.4, 1.7, 4.3, 14.5, and 48.5 $\mu g \cdot L^{-1}$). The mean water temperature was 25.3°C with a range from 23.5 to 26.8°C. Hatching success for controls averaged 74.9%, and ranged from 73.5 to 86.3% for the five exposure levels (Table 4). Hatching success was not affected by the dinoseb concentrations. The mean percent of fry that were abnormal (including dead) at time of transfer was 3.2% for controls, and ranged from 0.6 to 6.0% for the five levels of dinoseb exposure. None of the dinoseb exposures resulted in a significant increase ($p>0.05$) in abnormal newly hatched fry. Survival of transferred fry (30 per exposure chamber) through 60 days of exposure averaged 26.5 for controls and ranged from 2.5 to 26.0 for the five exposure levels. The dinoseb exposure replicates at 48.5 $\mu g \cdot L^{-1}$ averaged only two survivors, which was significantly less ($p<0.01$) than the survival of controls.

Wet weight at 60 days averaged 0.599 g for controls, and ranged from 0.517 to 0.733 g for the five exposure levels of dinoseb. The highest exposure (48.5 $\mu g \cdot L^{-1}$) caused a significant decrease ($p<0.01$) in weight. Increased growth occurred at all other exposures, and at 1.7 $\mu g \cdot L^{-1}$ a

TABLE 4. HATCHABILITY, ABNORMAL DEVELOPMENT, SURVIVAL AND GROWTH OF FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO DINOSEB FOR 64 DAYS

Parameter	Mean Dinoseb Concentration ± s.d. ($\mu g \cdot L^{-1}$)					
	0.0 ± 0.0 (n=19)	0.4 ± 0.2 (n=38)	1.7 ± 1.2 (n=39)	4.3 ± 0.6 (n=39)	14.5 ± 2.6 (n=39)	48.5 ± 7.8 (n=39)
Mean percent hatch[a]	74.9	85.0	86.3	80.0	73.5	79.7
Mean percent abnormal and dead[b]	3.2	0.6	3.4	3.5	6.0	1.5
Mean number of survivors at 60 days post-hatch[c]	26.5	23.5	24.5	26.0	16.0	2.5**
Mean wet weight at 60 days post-hatch (g)	0.599	0.683	0.733##	0.647	0.679	0.517**
Mean total length at 60 days post-hatch (mm)	39.7	41.3	42.2#	39.5	40.5	35.2

[a] Live fry/total eggs.

[b] Abnormal (deformed) + dead fry/total fry at time of transfer from egg cups (5 days after initial exposure of eggs).

[c] Based on 30 fry transferred to duplicate exposure chambers.

** Significantly different from controls ($p<0.01$).

Significantly longer than controls ($p<0.05$ from two-tailed Dunnett's test).

Significantly heavier than controls ($p<0.01$ from two-tailed Dunnett's test).

significant increase ($p<0.01$) in weight occurred. Mean length at 60 days was 39.7 mm for controls, and ranged from 35.2-42.2 mm for dinoseb-exposed fish. Dinoseb exposure did not significantly reduce ($p>0.05$) fish length, but at 1.7 $\mu g \cdot L^{-1}$ a significant length increase ($p<0.05$) was observed.

The "no effect" concentration for fathead minnows exposed to dinoseb was between 14.5 and 48.5 $\mu g \cdot L^{-1}$. This was based on the two most sensitive parameters of fry survival and wet weight at 60 days post-hatch.

Diuron

Fathead minnow eggs (83 to 104 total per incubation chamber) less than 24 hrs old were exposed to diuron at six duplicated control and exposure concentrations (means of 2.6, 6.1, 14.5, 33.4, and 78.0 $\mu g \cdot L^{-1}$). The mean water temperature was 25.0°C with a range from 23.8 to 27.0°C. Hatching success for controls averaged 67.9%, and ranged from 66.1 to 77.9% for the five exposure levels (Table 5). Hatching success was not affected by diuron exposure. The mean percent of abnormal (including dead) fry at time of transfer was 2.2% for controls, and ranged from 0.6 to 15.0% for the five diuron exposures. The highest concentration of 78.0 $\mu g \cdot L^{-1}$ resulted in a significant increase ($p<0.01$) in percent abnormal fry at hatch. Survival of transferred fry (30 per aquarium) averaged 24 for controls, and ranged from 8 to 28 for the five diuron exposures. The highest concentration (78.0 $\mu g \cdot L^{-1}$) resulted in a significant reduction ($p<0.05$) in survival through 60 days of exposure after hatching.

Wet weight at 60 days averaged 0.568 g for controls, and ranged from 0.496 g to 0.568 g for the five diuron exposures. Fry length at 60 days averaged 32.2 mm for controls, and ranged from 29.1 to 32.4 mm for the five exposures. Neither wet weight nor length were significantly affected ($p>0.05$).

TABLE 5. HATCHABILITY, ABNORMAL DEVELOPMENT, SURVIVAL AND GROWTH OF FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO DIURON FOR 64 DAYS

Parameter	Mean Diuron Concentration ± s.d. ($\mu g \cdot L^{-1}$)					
	0.0 ± 0.0 (n=18)	2.6 ± 0.7 (n=38)	6.1 ± 1.6 (n=36)	14.5 ± 2.0 (n=37)	33.4 ± 4.8 (n=38)	78.0 ± 8.1 (n=38)
Mean percent hatch[a]	67.9	77.9	75.0	71.8	67.9	66.1
Mean percent abnormal and dead[b]	2.2	0.6	1.3	3.7	8.2	15.0**
Mean number of survivors at 60 days post-hatch[c]	24.5	26.5	28.0	21.5	22.5	7.5*
Mean wet weight at 60 days post-hatch (g)	0.568	0.568	0.563	0.619	0.563	0.496
Mean total length at 60 days post-hatch (mm)	32.2	32.3	32.4	32.3	31.0	29.1

[a] Live fry/total eggs after 5 days.

[b] Abnormal (deformed) + dead fry/total fry at time of transfer from egg cups (5 days after initial exposure of eggs).

[c] Based on 30 fry transferred to duplicate exposure chambers.

* Significantly different from controls ($p<0.05$).

** Significantly different from controls ($p<0.01$).

The "no effect" concentration for fathead minnows exposed to diuron was between 33.4 and 78.0 $\mu g \cdot L^{-1}$. This estimate was based on the two parameters that were significantly affected at 78.0 $\mu g \cdot L^{-1}$. These were abnormal fry at time of transfer and survival through 60 days.

Propanil

Fathead minnow eggs (98 to 104 total per incubation chamber) less than 24 hrs old were exposed to propanil at six duplicated control and exposure concentrations (means of 0.4, 0.6, 1.2, 2.4, and 3.8 $\mu g \cdot L^{-1}$). The mean water temperature was 25.3°C with a range from 23.6 to 27.2°C. Hatching success averaged 75.9% for controls, and ranged from 56.6 to 80.5% for the five propanil exposures (Table 6). The highest exposure (3.8 $\mu g \cdot L^{-1}$) resulted in a 56.6% hatch, which was significantly lower ($p<0.05$) than the control hatch. Percent abnormal (plus dead) fry at the time of transfer from egg cups was 3.0% for controls, and ranged from 5.5 to 65.8% for the five propanil exposures. The concentration of 3.8 $\mu g \cdot L^{-1}$ resulted in a significant increase ($p<0.01$) in percent abnormal fry. The mean percent of surviving fry at 54 days post-hatch was 93.4% for controls, and ranged from 0.0 to 72.5% for the five propanil exposure levels. Exposures of 1.2 $\mu g \cdot L^{-1}$ and above resulted in a significant decrease ($p<0.01$) in survival. Survivorship curves for the five exposures are presented in Fig. 1.

Wet weight of fry at 54 days post-hatch averaged 0.590 g for controls, and ranged from 0.448 to 0.558 g for the three lowest propanil exposures. The higher two exposures (2.4 and 3.8 $\mu g \cdot L^{-1}$) had no survivors at 54 days. Wet weight was not affected at the three lower propanil exposures. Since many of the fish appeared swollen, the fish were oven-dried at 50°C for 24 hrs following wet weight measurements to determine if an edematous condition existed.

TABLE 6. HATCHABILITY, ABNORMAL DEVELOPMENT, SURVIVAL AND GROWTH OF FATHEAD MINNOWS (Pimephales promelas) EXPOSED TO PROPANIL FOR 58 DAYS

Parameter	Mean Propanil Concentration ± s.d. ($\mu g \cdot L^{-1}$)					
	0.0 ± 0.0 (n=13)	0.4 ± 0.3 (n=27)	0.6 ± 0.4 (n=27)	1.2 ± 0.7 (n=24)	2.4 ± 0.8 (n=14)	3.8 ± 0.4 (n=5)
Mean percent hatch[a]	75.9	80.5	70.2	63.4	64.0	56.6*
Mean percent abnormal and dead[b]	3.0	5.5	10.4	9.4	13.5	65.8**
Mean percent fry survival at 54 days post-hatch[c]	93.4	72.5	50.0	16.6**	0**	0**
Mean wet weight at 54 days post-hatch (g)	0.590	0.558	0.491	0.448	--	--
Mean dry weight at 54 days post-hatch (g)	0.152	0.132	0.119*	0.113	--	--
Mean total length at 54 days post-hatch (mm)	38.3	36.7	34.2**	33.1**	--	--

[a] Live fry/total eggs after 4 days of incubation.

[b] Abnormal (deformed) + dead fry/total eggs at time of transfer from egg cups (4 days after initial exposure of eggs).

[c] Based on mortality of 30 fish/chamber through day 30 post-hatch and 20 fish/chamber between days 30-54.

* Significantly different from controls ($p<0.05$).

**Significantly different from controls ($p<0.01$).

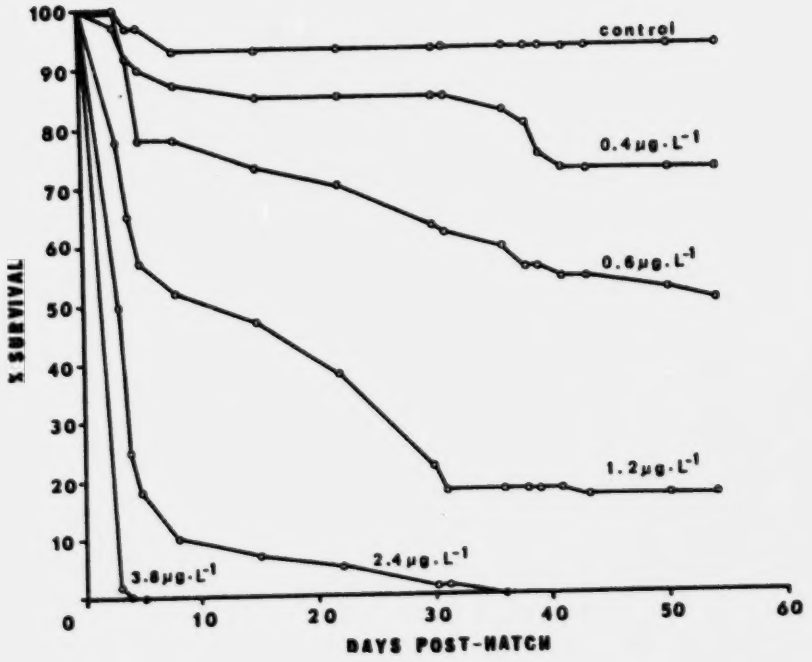


Figure 1. Survival curves for fry and juvenile fathead minnows (Pimephales promelas) exposed to propanil in an early life-stage toxicity test. Each curve represents a combination of fish from duplicate exposure chambers (n=60 at day 0).

The mean dry weight of control fish was 0.152 g. Fish exposed to 0.6 $\mu g \cdot L^{-1}$ averaged 0.119 g, a significant weight reduction ($p<0.05$). Fish exposed to 1.2 $\mu g \cdot L^{-1}$ propanil appeared still lighter (0.113 g), but because of the low number of surviving fish ($n=10$), this test did not reach significance at $p<0.05$. Mean length at 54 days was 38.3 mm for control fry, and ranged from 33.1 to 36.7 mm for fry from the three exposures. Fry length was significantly reduced ($p<0.01$) at propanil exposures of 0.6 and 1.2 $\mu g \cdot L^{-1}$. Every parameter tested, with the exception of wet weight, was adversely affected by propanil. The "no effect" concentration based on the two most sensitive parameters (dry weight and length at 54 days post-hatch) was between 0.4 and 0.6 $\mu g \cdot L^{-1}$.

Bioconcentration Tests

A summary of bioconcentration potential, metabolism, and elimination of the five herbicides in fathead minnows is presented in Table 7. The raw data were adjusted for recoveries of ^{14}C-labeled herbicides from both water and fish. Mean recoveries from water and fish, respectively, were: alachlor-102.6 and 86.8%, bromacil-87.5 and 88.1%, dinoseb-102.5 and 78.2%, diuron-93.2 and 91.9%, and propanil-92.8 and 90.8%.

Alachlor

Thirty-day old fathead minnows were exposed to ^{14}C-labeled alachlor at mean water concentrations of 0.66 and 9.95 $\mu g \cdot L^{-1}$ for 21 days. The mean water temperature was 24.9°C with a range from 23.8 to 25.8°C. Uptake of alachlor from water was rapid with an apparent equilibrium between water and tissue achieved in one day (Appendix C, Fig. C-1). Mean bioconcentration factors of ^{14}C equivalents in whole fish from days 1-21 were 50.2 and 41.4 for lower and

TABLE 7. BIOCONCENTRATION, METABOLISM AND ELIMINATION OF FIVE TEST HERBICIDES IN FATHEAD MINNOWS (*Pimephales promelas*)

Herbicide	Mean H_2O concentration ($\mu g \cdot L^{-1}$)	^{14}C Bio-concentration factor	Percent parent compound at end of uptake[a]	Percent depuration of ^{14}C in 24 hr	Percent depuration of ^{14}C at end of test (days of depuration in parentheses)
Alachlor	0.66	50.2	13.2	84.0	96.4 (14)
	9.95	41.4		78.3	99.8 (14)
Bromacil	4.25	2.8	nd[b]	nd	nd
	35.11	3.5		nd	nd
Dinoseb	0.62	61.5	2.3	67.4	94.6 (14)
	7.22	64.1		73.8	97.9 (14)
Diuron	3.15	157.0	1.3	83.8	98.7 (21)
	30.40	143.7		76.4	99.0 (21)
Propanil	0.34	69.0	1.8	80.0	96.4 (10)
	5.09	111.3		80.8	95.4 (10)

[a] Determined as portion of ether-extractable fraction that chromatographed by TLC as parent compound from fish exposed to higher concentrations of test herbicides.

[b] Not determined.

higher exposures, respectively (Table 7).

Elimination of alachlor was rapid, with 84.0 and 78.3% of the mean plateau tissue concentration lost during the first 24 hrs for fish exposed to lower and higher concentrations, respectively. Depuration was observed for 14 days, at which time 96.4 and 99.8% had been eliminated at lower and higher exposures, respectively. At the end of the uptake phase, 23.4% of total ^{14}C in fish tissue was extracted with ether. Of this soluble fraction, 56.5% chromatographed by thin layer chromatography (TLC) as parent compound, or 13.2% of the total tissue ^{14}C. Unextractable ^{14}C comprised 76.5% of the total ^{14}C, indicating a major amount of total ^{14}C was apparently in a "bound" form.

Bromacil

Thirty-day old fathead minnows were exposed to ^{14}C-labeled bromacil at mean water concentrations of 4.25 and 35.11 $\mu g \cdot L^{-1}$ for 17 days. Mean water temperature was 24.2°C and ranged from 23.0 to 25.5°C. Bromacil did not appreciably bioconcentrate in the fish. Mean bioconcentration factors of ^{14}C equivalents in whole fish from days 1-17 were 2.8 and 3.5 for lower and higher exposures, respectively (Table 7). The fish were not analyzed for metabolites.

Dinoseb

Fathead minnows (30-days old) were exposed to ^{14}C-labeled dinoseb for 24 days at mean water concentrations of 0.62 and 7.22 $\mu g \cdot L^{-1}$. The mean water temperature was 25.1°C with a range from 23.8 to 26.1°C. An upward trend in tissue ^{14}C was evident on days 21 and 24 of the uptake phase of the study (Appendix C, Fig. C-2). Consequently, at the end of 24 days of uptake, one-half of the fish at the higher concentration were placed into a depuration chamber and one-half were exposed for an additional 4 days with daily

samplings. The mean exposure level for this group over 28 days was 7.76 $\mu g \cdot L^{-1}$. After 28 days of exposure, depuration was also followed in these fish.

Uptake of dinoseb was rapid, with an equilibrium between water and fish achieved within 24 hrs. Mean ^{14}C bioconcentration factors for days 1-24 of exposure were 61.5 and 64.1 for lower and higher exposures, respectively (Table 7). The mean ^{14}C bioconcentration factor for the higher dinoseb exposure over 28 days was 56.2.

Elimination of ^{14}C from dinoseb-exposed fish was rapid, with 67.4 and 73.8% of the mean plateau tissue concentration lost within 24 hrs for fish exposed for 24 days to lower and higher concentrations, respectively. After 14 days of depuration, 94.6 and 97.9% had been eliminated for lower and higher exposures, respectively (Table 7). Elimination of ^{14}C from fish exposed to the higher concentration of dinoseb for 28 days was similarly rapid, with 80.2 and 95.9% eliminated after 24 hrs and 10 days, respectively.

At the end of dinoseb uptake, 27.9% of the total ^{14}C in fish tissue was ether-soluble. Of this soluble fraction, 8.1% chromatographed by TLC as parent compound, or 2.3% of the total tissue ^{14}C (Table 7). Unextractable ^{14}C comprised 72.1% of the total ^{14}C.

Diuron

Fathead minnows (30 days old) were exposed to ^{14}C-labeled diuron for 24 days at mean water concentrations of 3.15 and 30.40 $\mu g \cdot L^{-1}$. The mean water temperature was 24.8°C with a range from 23.8 to 25.8°C. Uptake was rapid, with an equilibrium between water and fish achieved within 24 hrs (Appendix C, Fig. C-3). Mean bioconcentration factors of ^{14}C diuron equivalents for lower and higher exposures were 157.0 and 143.7 times, respectively (Table 7).

Elimination of ^{14}C from diuron-exposed fish was rapid, with 83.8 and 76.4% of the mean tissue concentration at equilibrium eliminated within 24 hrs at lower and higher exposures, respectively. After 21 days in clean water, 98.7 and 99.0% of the ^{14}C had been eliminated at the lower and higher exposures, respectively.

At the end of diuron uptake, 15.0% of the total ^{14}C was ether-extractable of which 8.4% chromatographed as the parent compound. This constituted 1.3% of the total tissue ^{14}C (Table 7). Unextractable ^{14}C comprised 85.0% of the total ^{14}C.

Propanil

Fathead minnows (30 days old) were exposed to ^{14}C labeled-propanil in two separate tests. In the first test, fish were exposed to mean water concentrations of 3.96 and 53.49 $\mu g \cdot L^{-1}$ for a total of 21 days. However, during this test significant fish mortality occurred (14.5 and 16.4% in lower and higher exposures, respectively), and the test was repeated at lower concentrations.

In the second test, fish were exposed for 17 days to ^{14}C-labeled propanil at mean water concentrations of 0.34 and 5.09 $\mu g \cdot L^{-1}$. The mean water temperature for the test was 24.7°C with a range from 24.0 to 26.4°C. No mortalities or behavioral changes were observed in the test organisms. Uptake was rapid, with an equilibrium reached within 24 hrs (Appendix C, Fig. C-4). Bioconcentration factors of total ^{14}C were 69.0 and 111.3 for lower and higher exposures, respectively.

Elimination of ^{14}C was rapid. After 24 hrs in clean water, 80.0 and 80.8% of the mean tissue concentration at equilibrium had been eliminated for lower and higher exposures, respectively. After 10 days in clean water, 96.4

and 95.4% had been eliminated.

At the end of propanil uptake, 12.4% of the total ^{14}C was ether-extractable. From TLC analysis, 14.9% of the extractable fraction chromatographed as parent compound, or 1.8% of the total tissue ^{14}C (Table 7). Unextractable ^{14}C comprised 87.6% of the total ^{14}C.

Metabolite Characterization

Alachlor. Rainbow trout were injected with 1.0 μCi (0.16 mg) of alachlor. Most (83-94%) of the radioactivity was recoverd in tank water, of which 60% appeared to be parent compound. There were 3 polar metabolites of alachlor present in the water, and 2 polar metabolites in bile. The liver extract contained one or more components close to the TLC plate origin. No alachlor metabolites were recovered in sufficient quantity for GC/MS characterization.

Bromacil. Three trout were injected with 1.0 μCi of labeled bromacil. Over 95% of the ^{14}C label from injected bromacil appeared in the water within 24 hrs. The remainder was in the bile (1-3%), with traces in the blood and liver. Of the radiolabeled material in the water, 70-80% was parent compound as determined by TLC and GC/MS. The rest consisted of two or more polar metabolites that did not move from the origin during TLC, and were not further characterized. Plasma and liver extracts showed essentially the same pattern as the water. TLC of bile extracts revealed at least two polar components which were apparently not altered by treatment with glucuronidase. These bile metabolites were present in quantities too small to be characterized by GC/MS.

Dinoseb. Ninety percent of the herbicide injected into trout was recovered in the water, of which 50% appeared to be parent compound by TLC. There were at least 2 polar metabolites and 1 or 2 metabolites less polar than

dinoseb in the water. The metabolite profile was similar in bile except that the parent compound was not present. No metabolites were characterized by GC/MS.

Diuron. Tank water from diuron-injected trout contained over 90% of the radioactivity after 24 hrs. The tank water contained at least five components, including parent diuron. A polar compound (20-25% of the total amount) failed to move from the origin. Two additional components were present with R_f values less than or identical to parent diuron (R_f 0.4 to 0.5). Parent diuron represented 35-40% of the total radioactivity in the tank water. The fifth component appeared to be less polar than diuron (R_f 0.65 to 0.7), and represented 5-10% of the total radioactivity. The bile contained 5-6 radiolabeled derivatives of diuron.

Propanil. Each of 3 trout received 1 μCi (21 μgm) of propanil by intraperitoneal injection. Most of the injected dose was recovered in the water (75-108%) within 24 hr, and significant levels of radioactivity also occurred in the bile (9-22%). The liver contained 1-1.9% and less than 1% was recovered from blood fractions.

Three or more radioactive components were present in the water. The component with the greatest mobility (40-75% of the total radioactivity on the chromatogram) migrated with the same R_f (0.45-0.55) as propanil. A component of lower mobility (R_f=0.2) comprised 20-55% of the total radioactivity. The least mobile of the components (R_f<0.02) contained about 5% of the total activity.

An acetone extract of liver also contained 3 radioactive components with the same R_fs as the water components. In liver, however, the component with

the same R_f as propanil was in lower proportion (30-60%), and the most polar component in higher proportion (10%). The compound with intermediate mobility comprised 40-50% of the total in all 3 fish.

In bile, over 95% of the radioactivity was in the form of a highly polar compound or compounds (R_f<0.02), and 5% of the total was in a component with an R_f of 0.2. There was no trace in the bile of unchanged propanil. The highly polar component of bile was eluted from one chromatogram, and incubated with glucuronidase (from Helix pomatia). This treatment resulted in the formation of a less polar compound with an R_f of 0.16. With the addition of 1,4-saccharolactone (an inhibitor of glucuronidase) in the incubation mix, the metabolite was not altered. Thus, the highly polar bile metabolite appeared to be a glucuronide conjugate, but the aglycone was neither propanil nor 3,4-dichloroaniline from its TLC mobility.

For two herbicides, propanil and diuron, pre-exposure of the fish to unlabeled herbicide resulted in a different profile of metabolites, e.g. there were no labeled metabolites of propanil less polar than the parent compound in fish not pre-exposed to unlabeled herbicide, but there were 2-3 less polar labeled metabolites in fish pre-exposed to unlabeled propanil. The different profile may be due to enzyme induction or to the establishment of pools of metabolically active unlabeled metabolites with which the labeled metabolites equilibrate.

GC/MS Analysis of Metabolites. No herbicide metabolites were completely characterized by mass spectral analysis; however, some information on metabolites of propanil and diuron was obtained.

Propanil. Eight possible metabolites of propanil were submitted for

analysis, of which 2 were partially characterized. The second most polar compound on TLC was hydroxylated in the propionic acid moiety, and was, therefore, either 3',4'-dichloro-2-hydroxypropionanilide or 3',4'-dichloro-3-hydroxypropionanilide. Another metabolite tentatively identified as 3,4,3',4'-tetrachloroazobenzene (TCAB) based on mass spectral data was probably not this compound. Authentic TCAB was synthesized by a published method (Corbett and Holt, 1963) and was much less polar on TLC than the metabolite. The metabolite migrated with the same R_f as 3,4-dichloroaniline (DCA), and because DCA is a known photochemical precursor of TCAB, we tentatively concluded that the metabolite was DCA, and that TCAB was formed on the TLC plate during the interval between chromatography and mass-spectral analysis.

Diuron. GC and MS analysis of phenylurea herbicides is complicated by the thermal instability of these compounds. Tri-substituted ureas such as diuron pyrolyze to an aromatic phenylisocyanate and aliphatic component. Pyrolysis of mono- and di-substituted phenylureas results in the phenylisocyanate, the aliphatic component, and the corresponding aniline (Büchert and Lokke, 1975). We have used this information to interpret the mass spectra of diuron metabolites.

Four bile metabolites from diuron-exposed trout were submitted for analysis. Only the most polar was present in sufficient quantity to be detected by the mass spectrometer. The mass spectrum of the polar metabolite resembled that of diuron, implying that no ring alteration had occurred, nor had the N-1 position been dealkylated.

Three diuron metabolites were recovered from water, and submitted for analysis. The spectrum of the most polar metabolite suggested that demethylation of diuron had occurred. This was supported by the presence of DCA in

the GC profile. The metabolite with intermediate polarity migrated with the same R_f as reference diuron but its spectrum was much like the first metabolite indicating that at least one methyl group had been lost. DCA was also present in the GC profile of the second metabolite. The third diuron water metabolite migrated with DCA and mass spectral data confirmed that this compound was DCA.

Bromacil. Two possible metabolites of bromacil were recovered from exposure water and analyzed. One compound, which migrated with the bromacil standard was identified as the parent compound.

Alachlor and Dinoseb. Metabolites of these compounds were analyzed but there was insufficient material to generate mass spectra.

In Vitro Metabolism of Propanil

In several experiments on the in vitro hepatic conversion of propanil to metabolites there was a consistent pattern which resembled that seen in tank water of fish injected with the herbicide. About 5% of the total radioactivity was converted to metabolites, of which 50% was in a polar fraction which remained at the origin during TLC, and the remainder was in a fraction of intermediate polarity between propanil and the immobile fraction. With multiple chromatogram development, the polar fraction was resolved into three and possibly more components. The fraction of intermediate polarity was resolved into two components. Occasionally, there was the suggestion of a small band that migrated slightly faster than propanil.

Binding of Herbicides to Macromolecules

All of the herbicides exhibited time-dependent binding to macromolecules, with alachlor binding most extensively and propanil the least (Table 8). In

general, the binding was not dependent on NADPH, was not altered by the addition of glutathione, and was not altered by the substitution of heat-inactivated microsomes for active microsomes. The binding was thus apparently not dependent on mixed-function oxidase activity and may have been due solely to the affinity of the parent herbicides for protein and other macromolecules.

TABLE 8. BINDING OF HERBICIDES TO MACROMOLECULES

Herbicide	Nanomoles herbicide/gm protein
Alachlor	75.2
Bromacil	3.6
Dinoseb	13.2
Diuron	6.7
Propanil	2.3

Herbicide Stability in Solution

Declines in concentration with time occurred in the dinoseb and propanil solutions. Dinoseb declined by 21% in 40 days in Lake Superior water. Propanil declined curvilinearily during the 194 day test with an estimated half-life of 65 days. The principal breakdown product in the propanil solution was identified by GC/MS analysis as 3,4-dichloroaniline. Bromacil and diuron remained stable during the 40 day test. Alachlor was not tested for its stability in Lake Superior water.

GC/MS Analysis of Technical Grade Propanil and Diuron For Contaminants

GC/MS analysis of technical propanil used in this study revealed the presence of 0.67 $mg \cdot g^{-1}$ of 3,3',4 ,4'-tetrachloroazobenzene (TCAB). No 3,3',4,4'-tetrachloroazoxybenzene (TCAOB) was present above the detection limit of 30 $\mu g \cdot g^{-1}$. No TCAB was found in technical grade diuron at a detection limit of 10 $\mu g \cdot g^{-1}$, nor was any TCAOB present above the 30 $\mu g \cdot g^{-1}$ detection limit. GC/MS printouts of reconstructed ion chromatograms and mass chromatograms for standards of TCAB and TCAOB, and for samples of technical grade propanil and diuron are presented in Appendix D (Figs. D-1 to D-3).

SECTION VI

DISCUSSION

Acute Toxicity

Static exposures of rainbow trout and bluegills to technical grade alachlor of 100% purity resulted in 96 hr LC_{50} values of 2.4 and 4.3 $mg \cdot L^{-1}$, respectively; while exposure to a 43% liquid formulation of alachlor resulted in LC_{50} values of 1.4 and 3.2 $mg \cdot L^{-1}$, respectively (Johnson and Finley, 1980). In another report that did not specify test conditions, 96 hr median tolerance limits (TL_m's) for rainbow trout and bluegills were 2.3 and 13.4 $mg \cdot L^{-1}$, respectively, for a 4 lb/gal emulsifiable concentrate of alachlor (Weed Science Society of America, 1970). The 96 hr LC_{50} for fathead minnows in the present study was 5.0 $mg \cdot L^{-1}$.

One account of bromacil toxicity toward fish was found (U.S. Environmental Protection Agency, 1975), which had originated through a personal communication with E.I. duPont de Nemours and Co., Inc. TL_m values based on an 80% bromacil formulation for bluegill sunfish were 103 and 71 $mg \cdot L^{-1}$ at 24 and 48 hr, respectively. For rainbow trout TL_m values were 102, 75, and 38 $mg \cdot L^{-1}$ at 24, 48, and 72 hr, respectively; and for carp (Cyprinus carpio), 164 $mg \cdot L^{-1}$ at both 24 and 48 hr. No information was provided on the nature of the exposures. In the present study, LC_{50} values of 185, 183, and 182 $mg \cdot L^{-1}$ were obtained for fathead minnows at 24, 48, and 96 hr, respectively. Bromacil was the least

toxic of the five herbicides studied in acute exposures.

The 24, 48, and 96 hr LC_{50} values for dinoseb with fathead minnows in the present study were 0.8, 0.7, and 0.7 $mg \cdot L^{-1}$, respectively. Alabaster (1969) reported 24 and 48 hr LC_{50}s of 3.4 and 3.0 $mg \cdot L^{-1}$, respectively, for dinoseb (90% active ingredient) and harlequin fish (Rasbora heteromorpha) under toxicant replacement conditions in hard water (250 $mg \cdot L^{-1}$ as $CaCO_3$, pH 7.2, 20°C). Dinoseb-exposed mosquitofish (Gambusia affinis) in static bioassays using dechlorinated tap water at 21°C had 24 hr LC_{50} values of 0.87 and 0.96 $mg \cdot L^{-1}$ in insecticide-susceptible and insecticide-resistant populations, respectively (Fabacher and Chambers, 1974).

Lipschuetz and Cooper (1961) obtained a 24 hr LC_{50} of 0.24 $mg \cdot L^{-1}$ in a flow-through test (pH 8.0, 21°C) with blacknose dace (Rhinichthys atratulus). The same authors found pH to greatly affect the toxicity of dinoseb to rainbow trout. For rainbow trout tested at 18.3°C, 24 hr LC_{50} values of 0.073 (pH 6.9) and 0.30 (pH 8.0) $mg \cdot L^{-1}$ were obtained. Similarly, Woodward (1976) found decreased pH resulted in increased toxicity of dinoseb to cutthroat (Salmo clarki) and lake trout (Salvelinus namaycush). Dinoseb toxicity also increased with increased water temperature and water hardness, but not as greatly as with decreased pH. LC_{50} values (96 hr) of 1.35, 0.13, and 0.041 $mg \cdot L^{-1}$ were reported for cutthroat trout at pHs of 8.5, 7.5 and 6.5, respectively, at 10°C in standard reconstituted soft water. Lake trout LC_{50} values were 1.40, 0.77, and 0.32 $mg \cdot L^{-1}$ at pHs of 8.5, 7.5, and 6.5, respectively. Woodward (1976) stated that ionization of dinoseb (a weak acid with pKa = 4.4) at higher pHs could decrease its ability to be transported across the gill, and therefore reduce its toxicity. This interpretation of the effect of pH on toxicity is supported by other reports (Maren et al., 1968; Hunn and Allen, 1974) which

state that ionized forms of molecules penetrate gill membranes less readily than nonionized forms.

In another study with cutthroat and lake trout in which fish were exposed statically at 10°C, 96 hr LC_{50} values for dinoseb were 0.067 and 0.044 $mg \cdot L^{-1}$, respectively (Johnson and Finley, 1980). A temperature increase from 10° to 15°C resulted in no substantial change in toxicity to either species. A decrease in temperature from 10° to 5°C did not affect the toxicity to cutthroat trout, but LC_{50}s were 3 times higher for lake trout. Increased pH again decreased the toxicity of dinoseb. Aging of test solutions caused a twofold increase in LC_{50} values after 1 week, but no change after 4 weeks.

A 96 hr dinoseb lethal threshold concentration of 0.070 $mg \cdot L^{-1}$ was determined for juvenile Atlantic salmon (Salmo salar) in a static test regime (Zitko et al., 1976). The lethal threshold was determined by calculating the geometric mean of the lowest concentration at which median mortality was observed, and the highest concentration at which no mortality occurred.

Diuron LC_{50} values in the present study with fathead minnows were 23.3, 19.9, and 14.2 $mg \cdot L^{-1}$ at 24, 48, and 96 hr, respectively. From a review of diuron acute toxicity tests with fish, 96 hr LC_{50} values generally ranged between 1 and 25 $mg \cdot L^{-1}$ for most species (Johnson and Julin, 1974). However, the 96 hr LC_{50} values ranged from 0.5 $mg \cdot L^{-1}$ for larval striped bass (Morone saxatilis) (Hughes, 1973) to the solubility limit (42 $mg \cdot L^{-1}$ at 25°C) for other species (Bond et al., 1960). A reiteration of the forementioned review with 96 hr LC_{50} data is presented in Table 9. The acute toxicity of diuron was not greatly affected by pH or hardness, but was decreased in aged solutions (Fish-Pesticide Research Laboratory, 1974; Johnson and Finley, 1980). Increased water temperature increased diuron toxicity to bluegills in one study (Macek et

TABLE 9. 96 HR ACUTE TOXICITY OF DIURON TO VARIOUS FISH SPECIES

Species	96 hr LC_{50} ($mg \cdot L^{-1}$)	Formulation	Temp (°C)	pH	Alkalinity ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Comments	Reference
Coho salmon (*Oncorhynchus kisutch*)	1-10	Technical (95% a.i.)	13	7.4	35	40	Continuous exposure	Fish-Pesticide Research Laboratory (1974)
Lake trout (*Salvelinus namaycush*)	2.5	"	10	6.5	38	33	" "	"
	2.7	"	10	7.0	38	33	" "	"
	2.4	"	10	7.5	38	33	" "	"
	2.5	"	10	8.5	38	33	" "	"
	2.5	"	5	7.5	112	104	" "	"
	1.2	"	15	7.5	112	104	" "	"
	3.2	"	10	7.0	41	33	Solution aged 1 wk	"
	3.6	"	10	7.0	41	33	Solution aged 3 wks	"
	11.5	"	10	7.0	41	33	Solution aged 3 wks	"
Rainbow trout (*Salmo gairdneri*)	3.5	"	12	7.5	35	40	Solution not aged	"
	4.2	"	12	7.5	35	40	Solution aged 1 wk	"
	7.4	"	12	7.5	35	40	Solution aged 3 wks	"
	9.4	"	12	7.5	35	40	Solution aged 4 wks	"
Fathead minnow (*Pimephales promelas*)	32	80% wp	-	-	-	-	Continuous exposure, non-toxic to 32 $mg \cdot L^{-1}$	Wile (1968)
Brown bullhead (*Ictalurus nebulosus*)	11	"	-	-	-	-	Continuous exposure	Walker (1965)
Channel catfish (*Ictalurus punctatus*)	38	80% wp	17	7.5	250	-	Continuous exposure	Dayfield (1971)
Bluegill (*Lepomis macrochirus*)	10.4	Technical (95% a.i.)	12	7.5	35	40	Continuous exposure, fingerling fish (0.5-1.5g)	Fish-Pesticide Research Laboratory (1974)

(Continued)

TABLE 9 (continued)

Species	96 hr LC_{50}-1 ($mg \cdot L^{-1}$)	Formulation	Temp (^{o}C)	pH	Alkalinity ($mg \cdot L^{-1}$)	Hardness ($mg \cdot L^{-1}$)	Comments	Reference
Bluegill (Lepomis macrochirus)	8.5	80% wp	-	-	-	-	Continuous exposure, fingerling fish; 2"-3" size	Walker (1965)
	25	"	-	-	-	-	4"-5" size	"
	9.1	Technical (95% a.i.)	7	7.1	35	40	Continuous exposure	Macek et al., (1969)
	8.9	"	13	7.1	35	40	" "	"
	7.6	"	18	7.1	35	40	" "	"
	5.9	"	24	7.1	35	40	" "	"
	2.1	"	30	7.1	35	40	" "	"
	10.0	"	12	8.0	235	44	" "	Fish Pesticide Research Laboratory (1974)
	8.3	"	12	8.0	235	170	" "	"
	8.0	"	12	8.0	235	300	" "	"
	10.2	"	12	6.5	35	40	" "	"
	10.4	"	12	8.5	35	40	" "	"
	7.0	"	12	9.5	35	40	" "	"
Smallmouth bass (Micropterus dolomieui)	19	80% wp	-	-	-	-	Test conducted with fry and tap water	Wile (1968)
Largemouth bass (Micropterus salmoides)	42	-	20	7.4-7.7	41-71	-	Continuous exposure	Bond et al., (1960)
Striped bass (Morone saxatilis)	0.5	80% wp	21	-	-	-	Continuous exposure, larval fish	Hughes (1973)
	6.0	"	21	-	-	-	Continuous exposure, fingerling fish	"

al., 1969), but had little effect upon trout or bluegills in another study (Johnson and Finley, 1980).

Propanil LC_{50}s with fathead minnows in the present study were 11.5, 10.2, and 8.6 $mg \cdot L^{-1}$ at 24, 48, and 96 hr, respectively. In the mosquitofish, static propanil exposures produced LC_{50} values of 11.21, 8.45, and 7.62 $mg \cdot L^{-1}$ at 24, 48, and 72 hr, respectively; and in the green sunfish (Lepomis cyanellus), LC_{50} values of 9.10, 8.26, and 5.85 $mg \cdot L^{-1}$ at 24, 48, and 72 hr, respectively (Davey et al., 1976). They used well water of unspecified hardness or pH, and a propanil formulation of 3 $lb \cdot gal^{-1}$, effective concentration. A static exposure with a 3 $lb \cdot gal^{-1}$ liquid formulation of propanil in aged tap water of unspecified character yielded mosquitofish TL_m values of 11.30, 11.00, 10.17, and 9.46 $mg \cdot L^{-1}$ at 24, 48, 72, and 96 hr, respectively (Chaiyarach et al., 1975). In a brief account, propanil at 20 $mg \cdot L^{-1}$ in continuously flowing dechlorinated tap water produced 16.6% mortality at 24 hr with young channel catfish (Ictalurus punctatus) averaging 6.8 g in weight (Chambers and Fabacher, 1974). A 96 hr LC_{50} value of 3.8 $mg \cdot L^{-1}$ was obtained for fingerling channel catfish in a static test (McCorkle et al., 1977).

The order of acute toxicity to fathead minnows of the five herbicides studied after 24 to 192 hr of exposure was: dinoseb>alachlor>propanil>diuron> bromacil. LC_{50} concentrations ranged from 0.4 to 185 $mg \cdot L^{-1}$ for these compounds over this exposure period. Such concentrations would not likely be encountered in streams or lakes from field runoff.

Early Life-Stage Toxicity

Chronic (long-term) laboratory exposures often include concentrations of toxicants which might be encountered in the field. These exposures may also extend over a complete life cycle or at least over the most sensitive life-

stages. Such tests may help predict potentially harmful ecological effects (Mawdesley-Thomas, 1971).

Several studies with selected toxicants and fish species have demonstrated that early life-stages are the most sensitive (Benoit, 1975; Eaton, 1974). Egg and fry (30-60 day-old) bioassays provided good estimates of chronic toxicity with bluegills (Lepomis macrochirus) exposed to cadmium when compared to complete life-cycle exposures (Eaton, 1974). In a review of 56 life-cycle toxicity tests with 34 chemicals and four fish species, the embryo-larval and early juvenile life-stages were the most, or among the most, sensitive (McKim, 1977). In 82% of the cases, the maximum acceptable toxicant concentration (MATC) estimated by embryo-larval or early juvenile exposures was identical to the MATC established by the longer, more involved, and more costly partial or complete life-cycle toxicity tests. Additional tests with several fish species and selected metals (copper and cadmium) substantiated the observation that embryo-larval tests reliably estimate complete life-cycle chronic tests (Eaton et al., 1978; McKim et al., 1978).

In the present study, herbicide exposures extended from the fertilized egg stage through 54-60 days post-hatch. For alachlor, the early exposure parameters (% hatch, % abnormal and dead fry at time of transfer from egg cups) were not significantly affected. However, length and weight of juvenile fish at 60 days post-hatch were affected, with a highly significant reduction ($p<0.01$) in weight and length at the highest exposure of 1.10 $mg \cdot L^{-1}$ (Table 2). Fish weight and length parameters were also reduced ($p<0.05$) at the concentration of 0.14 $mg \cdot L^{-1}$. However, since there were no significant effects at the next two higher exposures of 0.26 and 0.52 $mg \cdot L^{-1}$, the "no-effect" estimate was considered to be between 0.52 and 1.10 $mg \cdot L^{-1}$. Concentrations in this range would not likely

be encountered from field run-off into streams and lakes.

Length and weight at 60 days post-hatch were the parameters significantly affected by bromacil (Table 3). Weight was reduced ($p<0.05$) at all exposures ranging between 1.0 and 29.0 $mg \cdot L^{-1}$, while length was significantly reduced ($p<0.05$) at all exposures except 1.9 $mg \cdot L^{-1}$. The "no effect" concentration was below the lowest test concentration of 1.0 $mg \cdot L^{-1}$. However, in view of the low order of the acute toxicity of bromacil to fish and the "clean" nature of the diluter tubes leading to the exposure chambers (i.e., lack of algal growth), it was felt that the reductions in length and weight of bromacil-exposed fish may have been due partly to its algacidal properties in the exposure system. Young fathead minnows feed upon algae under natural conditions (Eddy and Underhill, 1976). Thus, although all exposure levels as well as the controls were fed equal volumes of brine shrimp, the controls may have had the additional advantage of an imported algal community as a dietary supplement.

Dinoseb did not affect egg hatch nor the incidence of abnormalities or mortalities among newly hatch fathead minnow fry (Table 4). Dinoseb at a concentration of 10.0 $\mu g \cdot L^{-1}$ caused a 24% decrease in the survival of lake trout (Salvelinus namaycush) alevins through the yolk absorption stage (Woodward, 1976); and reduced fry survival over the period from yolk absorption to 60 days post-hatch to 22% compared to 86% for controls. In the present study, fathead minnow fry survival at 60 days post-hatch was not significantly affected by dinoseb exposures up to 14.5 $\mu g \cdot L^{-1}$; however, the 14.5 $\mu g \cdot L^{-1}$ exposure decreased the number of survivors to a mean of 16.0 out of 30 individuals, compared to 26.5 for controls. At 48.5 $\mu g \cdot L^{-1}$ of dinoseb, the number of survivors was decreased to a mean of 2.5 individuals out of 30, which was significant ($p<0.01$).

Length of fathead minnows through 60 days post-hatch was not significantly

reduced by any of the dinoseb exposures; however, weight was significantly reduced ($p<0.01$) at the highest exposure of 48.5 $\mu g \cdot L^{-1}$. Weight and length of lake trout fry at 60 days post-hatch were significantly reduced ($p<0.05$) by dinoseb exposures of 0.5 $\mu g \cdot L^{-1}$ and greater (Woodward, 1976). The rate of yolk absorption of lake trout fry exposed to dinoseb also decreased at the same exposure levels.

The "no-effect" concentration of dinoseb for lake trout was less than the lowest exposure level of 0.5 $\mu g \cdot L^{-1}$, based on the parameters of length and weight (Woodward, 1976). The "no-effect" concentration of dinoseb for fathead minnows in our study was between 14.5 and 48.5 $\mu g \cdot L^{-1}$, based upon the parameters of fry survival and fry weight through 60 days post-hatch. These "no-effect" levels are lower than the time-independent LC_{50} values ($TILC_{50}$) of 54 and 102 $\mu g \cdot L^{-1}$ for lake and cutthroat trout, respectively (Johnson and Finley, 1980). $TILC_{50}$ values are mathematically derived toxicant concentrations at which 50% of the test animals would be expected to survive indefinitely upon exposure to toxicants under flow-through conditions for up to 30 days. However, $TILC_{50}$ estimates would not encompass exposure to the early life-stages of the embryo and newly hatched fry. A cumulative toxicity index (96 hr $LC_{50}/TILC_{50}$) of 1.5 was reported for both cutthroat and lake trout, indicating little cumulative toxic action of dinoseb (Johnson and Finley, 1980).

Dinoseb has a water solubility of approximately 50 $mg \cdot L^{-1}$ (Agriculture Canada, 1973, Weed Society of America, 1970). While no information was found on watershed concentrations of dinoseb, several studies have been conducted on the herbicide atrazine, which has a water solubility of 33 $mg \cdot L^{-1}$ (Wauchope, 1978), slightly less than that of dinoseb. A comparison of the two herbicides may be useful, since dinoseb was degraded quite slowly in water in our study and in other

toxicity studies (Woodward, 1978; Johnson and Finley, 1980), although atrazine is more persistent in soil (Klingman and Ashton, 1975). Atrazine residues were commonly detected in Iowa surface waters (Richard et al., 1975), with concentrations as high as 42 $\mu g \cdot L^{-1}$ occurring in June. The overall June mean from three different surface waters was 4.9 $\mu g \cdot L^{-1}$. Atrazine residues in a Southwestern Ontario watershed also reached their highest concentrations in June, with a mean monthly concentration of 3.6 $\mu g \cdot L^{-1}$ (Roberts et al., 1979).

The highest concentrations of atrazine residues in surface waters were related to rainfall events, the severity of such events, and the proximity of these events to herbicide application (Richard et al., 1975; Roberts et al., 1979). In the case of dinoseb, with a slightly greater water solubility than atrazine, it may be possible for surface water residue loads to develop similar to those for atrazine, dependent upon variables such as application timing and rate, nature of the watershed and portions treated, rainfall timing, and rainfall severity.

If, in certain areas, the surface water dinoseb concentrations did, indeed, approximate the residue concentrations of atrazine (i.e. 1-5 $\mu g \cdot L^{-1}$, with occasional heavier loadings), dinoseb concentrations might periodically reach levels above 14.5 $\mu g \cdot L^{-1}$ and adversely affect those aquatic organisms with sensitivities to dinoseb similar to or greater than that of fathead minnows. Such water concentrations of dinoseb would certainly appear to have the potential to cause adverse effects upon more sensitive aquatic forms such as the salmonids, where the MATC was below 0.5 $\mu g \cdot L^{-1}$ for lake trout (Woodward, 1976). Studies are needed on surface water concentrations of dinoseb, especially from selected areas where it is extensively used.

Diuron affected two parameters in the fathead minnow embryo-larval test

at the highest exposure concentration of 78.0 $\mu g \cdot L^{-1}$. There was an increased incidence ($p<0.01$) in percent abnormal and dead fry at the time of transfer from egg cups to exposure chambers, and a decrease ($p<0.05$) in number of survivors (mean of 7.5) after 60 days of exposure. Based on these parameters, the "no-effect" concentration was between 33.4 and 78.0 $\mu g \cdot L^{-1}$. Flow-through tests produced $TILC_{50}$ values of 140 and 500 $\mu g \cdot L^{-1}$ for rainbow and cutthroat trout, respectively (Johnson and Finley, 1980). As with dinoseb, $TILC_{50}$ estimates were higher than our "no-effect" range for fathead minnows. Cumulative toxicity indices were 12.3 and 3.7 for rainbow and cutthroat trout, respectively, indicating a moderate degree of cumulative toxic action in rainbow trout (Johnson and Finley, 1980).

The growth of bluegill fingerlings in ponds treated with one application of diuron at concentrations ranging from 0.5 to 3.0 $mg \cdot L^{-1}$ was less than that of controls over a 6-month period (McCraren et al., 1969). This may have been partly due to oxygen stress, as low oxygen levels persisted for several days following diuron application with the resultant destruction of vascular plants.

Diuron has a water solubility of 42 $mg \cdot L^{-1}$ (Wauchope, 1978; Weed Sci. Soc. of Amer., 1970). Diuron, like atrazine, is an herbicide which provides full season weed control (Klingman and Ashton, 1975). However, it is more persistent in soil than atrazine, with a half-life of 7.0 months at 15°C as compared to 6.0 months for atrazine (Freed and Haque, 1973). In one study (Khan et al., 1976), phytotoxic diuron residues were detected in the soil 3 years after the last of a 7 year series of applications. Annual rates of diuron breakdown range from 30% to approximately 80% (Leonard et al., 1974; Dawson et al., 1968; Khan et al., 1976). Dissipation is mainly dependent upon microbial activity and not on chemical degradation (Majka and Lavy, 1977; Hill et al., 1955; McCormick and

Hiltbold, 1966; Murray et al., 1969; Sheets, 1964). Diuron has relatively low downward mobility in soil (Majka and Lavy, 1977; Khan et al., 1976), allowing for the compound to remain near the soil surface.

Diuron regularly appeared in streams and in sediments of streams and estuaries in Hawaii, where such streams received runoff from pineapple and sugarcane fields (Green et al., 1977). Diuron concentrations in estuarine waters ranged from 0.1 to 1 $\mu g \cdot L^{-1}$. In another field plot study, less than 0.12% of the annual application of diuron to cotton was lost as runoff (Willis et al., 1975). Their data suggested that with proper use on agricultural land in the lower Mississippi River Valley, diuron posed little threat to adjacent aquatic areas.

Diuron residues were detectable in water for 21 days in ponds treated at rates of 0.5 and 3.0 $mg \cdot L^{-1}$, and in plants and mud for 95 and 122 days, respectively (McCraren et al., 1969). The highest water residue concentration of 35 $\mu g \cdot L^{-1}$ was reported on day 21 in a pond treated with 3.0 $mg \cdot L^{-1}$ diuron. More information is needed on water concentrations of diuron from agricultural areas where it is extensively used to determine its potential for adversely affecting aquatic life at low exposure concentrations.

Propanil adversely affected all parameters studied, with the exception of juvenile fish wet weight at 54 days, at mean concentrations of 0.6 $\mu g \cdot L^{-1}$ and above in the present investigation. Dry weight of the survivors was significantly reduced at a concentration of 0.6 $\mu g \cdot L^{-1}$. The "no-effect" estimate for propanil was between 0.4 and 0.6 $\mu g \cdot L^{-1}$.

The observation that wet weight was not significantly affected by propanil exposure while dry weight was at 0.6 $\mu g \cdot L^{-1}$, in combination with observations of grossly swollen fry in propanil-exposed groups suggested that this herbicide

caused a water imbalance in the fish. Physiological studies to determine if and how propanil affects excretion or osmotic regulation would be of interest.

In some of the swollen fry reddish patches were visible through the skin in the lateral abdominal areas, which appeared to have been due to localized hemorrhaging or abnormal condition of the blood vessels. In a 60-day pond exposure to propanil at a single application rate of 7 $kg \cdot ha^{-1}$, propanil caused a hemolytic effect in immature ides or roaches starting on day 10 (Popova, 1973). Livers of propanil-exposed ides on day 10 had overfilled blood vessels, many with heomolyzed erythrocytes; and had focal blood discharges and disaggregation of liver cells.

In the field study by Popova (1973), immature ides were placed into control and exposure ponds one week after propanil application. Fish growth was considerably less in the treated pond after 60 days, as live weight gains of 63 and 14% were noted in the control and treatment ponds, respectively. Fish loss was 20%. Morphophysiological characteristics of propanil-exposed fish were changed, and did not return to normal even though fish were maintained for a long time in water free of propanil and its metabolites.

The solubility of propanil in water has been reported as 200 $mg \cdot L^{-1}$ (Gordon et al., 1964) and 500 $mg \cdot L^{-1}$ (Weed Society of America, 1970; Bailey and White, 1965). Propanil has been found to dissipate rapidly from rice paddy water. When propanil was applied to a rice field at the normal application rate of 3.4 $kg \cdot ha^{-1}$ in an impounded system, propanil concentrations of approximately 680 and 60 $\mu g \cdot L^{-1}$ were measured at the time of flooding and 24 hr later, respectively; while in a flowing system, propanil concentrations were approximately 400 and 50 $\mu g \cdot L^{-1}$ at 0 and 24 hrs, respectively (Deuel et al., 1977). Thus, propanil concentrations in the water were reduced by approximately

90% in one day. The dissipation of propanil in the flood water corresponded to the appearance of 3,4-dichloroaniline (Deuel et al., 1977). The principal breakdown product in Lake Superior water from our study was also identified as 3,4-dichloroaniline.

In another rice field study at a single propanil application of 7 $kg \cdot ha^{-1}$ (Popova, 1973), the propanil concentration in water was 200 $\mu g \cdot L^{-1}$ after 1 day and approximately 50 $\mu g \cdot L^{-1}$ for the subsequent 5 days. At 10-15 days, only metabolites were observed in the water. On days 20 and 60 no herbicide was observed in water, bottom sediments, or flora.

This study has shown that low levels of propanil adversely affect fish hatchability, the normal development and survival of newly hatched fry, and the growth and survival of juvenile fish. Even though propanil has been shown to dissipate quite rapidly under field conditions (Deuel et al., 1977; Popova, 1973), residues in the water during the first week after application may be sufficiently high to adversely affect fish populations if rice paddy flood water is inhabited by fish or if it passes over fish spawning and nursery grounds.

Several other fish chronic or early life-stage toxicity studies with herbicides have been reported. "No-effect" ranges for fathead minnows exposed to trifluralin and acrolein were 1.95 - 5.1 $\mu g \cdot L^{-1}$ and 11.4 - 41.7 $\mu g \cdot L^{-1}$, respectively (Macek et al., 1976). Picloram affected lake trout (Salvelinus namaycush) fry growth at the lowest concentration tested (35 $\mu g \cdot L^{-1}$), thus placing the "no-effect" range below this value (Woodward, 1976). Macek et al. (1976) determined a "no-effect" range for atrazine-exposed bluegills (Lepomis macrochirus) of 90 - 500 $\mu g \cdot L^{-1}$ based on equilibrium loss, and for atrazine-exposed fathead minnows of 210 - 870 $\mu g \cdot L^{-1}$ based on fry mortality. A "no-

effect" range of 300 - 1500 $\mu g \cdot L^{-1}$ was obtained in a study with 2,4-D (butoxyethanol ester) and fathead minnows (Mount and Stephan, 1967).

All five herbicides studied apparently inhibit plant photosynthesis either by affecting the electron transport process within the chloroplasts or by acting as inhibitory uncouplers of photophosphorylation in both chloroplasts and mitochondria (Moreland, 1980). In addition to these primary mechanisms of action within the chloroplasts and mitochondria, some of the herbicides studied are also known to produce secondary effects in plants (Moreland, 1980). Bromacil, dinoseb, and diuron interfere with cell membrane permeability and integrity. Bromacil and diuron also interfere with cell membrane lipid synthesis. Dinoseb inhibits DNA, RNA or protein synthesis.

Growth reduction of herbicide-exposed fish may have resulted from some of these same mechanisms of action having occurred within the cells of the fish. Moreland (1980) stated that plant and animal mitochondria are usually affected similarly by inhibitors and uncouplers, but with different degrees of sensitivities.

Uptake, Metabolism and Elimination

The five herbicides of the present study did not appreciably accumulate in fish tissue. Quantities of total ^{14}C were present in fish tissue at concentrations slightly greater than water concentrations for alachlor (45.8 x), dinoseb (62.8 x), diuron (150.4 x) and propanil (90.2 x); while for bromacil the total ^{14}C concentrations were similar in both fish tissue and water (total ^{14}C bioconcentration factor of 3.2 x). The percentages of total ^{14}C present in fish tissue determined to be parent herbicide at the end of the uptake phase were 13.2, 2.3, 1.3, and 1.8% for alachlor, dinoseb, diuron, and propanil,

respectively. This was not determined for bromacil due to the low level of total ^{14}C activity. From the contributions of the parent herbicides toward total ^{14}C activity in fish tissue, parent herbicide bioconcentration factors were: alachlor (6.0 x), bromacil (<3.2 x), dinoseb (1.4 x), diuron (2.0 x), and propanil (1.6 x).

Most herbicides that have been studied do not have a strong potential for tissue accumulation in fish. Negligible bioconcentration factors were obtained for 2,4-D dimethylamine salt (Sikka et al., 1977), atrazine (Macek et al., 1976), and simazine (Rodgers, 1970). However, trifluralin bioconcentrated in fish approximately 1,000 times the water exposure level (Macek et al., 1976). By contrast, many of the organochlorine pesticides or their metabolites (p,p'-DDT, p,p'-DDE, chlordane, hexachlorobenzene, mirex, dieldrin, toxaphene, heptachlor, heptachlor epoxide, methoxychlor, and endrin) were found to accumulate in fish from 4,500 to 51,000 times their concentrations in water (Veith et al., 1979; Hamelink et al., 1971; Jarvinen and Tyo, 1978).

Alachlor appeared to be readily eliminated as parent compound and also quite readily metabolized by rainbow trout, although the metabolites were not recovered in sufficient quantity for GC/MS characterization. In a model ecosystem study (Yu et al., 1975), alachlor was degraded to numerous unidentified polar metabolites.

Bromacil was rapidly eliminated from rainbow trout, mainly as parent compound. Two or more polar metabolites were produced, but were present in insufficient quantity to be characterized by GC/MS. Gardiner et al. (1969) found the primary urinary metabolite of bromacil in the rat to be a conjugate of 5-bromo-3-sec-butyl-6-hydroxymethyl uracil, and that bromacil was also hydroxylated at the 2 and 3 positions of the sec-butyl side chain. Some

debrominated metabolites were also found.

Dinoseb was readily eliminated as parent compound and also readily metabolized by rainbow trout. At least two polar metabolites and one or two metabolites less polar than dinoseb were present in the water of dinoseb-injected trout. A similar metabolite profile was observed in bile, except that no parent compound was present. However, no metabolites were characterized by GC/MS. Rats and mice readily metabolized dinoseb to a variety of products (Bandal and Casida, 1972). Glucuronic acid and sulfate were conjugated to the phenolic hydroxyl, and metabolites with either of the side chain methyl groups oxidized to carboxyls were found. The rat was able to reduce one of the nitro groups and to acetylate the resulting amino group. The mouse was unable to reduce either of the nitro groups. More than a dozen minor metabolites were present but were not characterized in that study (Bandal and Casida, 1972). Rabbits also produced a propionic acid metabolite and a glucuronide containing a reduced nitro group (Ernst, 1967).

Diuron was readily eliminated both as parent compound and as metabolites in rainbow trout injected with ^{14}C-labeled diuron. A metabolite present in the bile in sufficient quantity to be partially characterized by GC/MS had a mass spectrum resembling that of diuron, implying that neither ring alteration nor dealkylation in the N-1 position had occurred. Metabolites recovered from the water included 3,4-dichloroaniline and two different but unidentified demethylated products.

In man, diuron ingestion produced urinary metabolites identified as 1-(3,4-dichlorophenyl)-3-methyl urea, 1-(3,4-dichlorophenyl) urea, and 3,4-dichloroaniline (Geldmacher et al., 1971). Bohme and Ernst (1965) found N-(3,4-dichlorophenyl)-urea and N-(2-hydroxy-4,5-dichlorophenyl) urea to be the

predominant urinary metabolites in rats fed diuron. Dogs fed diuron on a chronic basis produced demethylated urinary metabolites of N-(3,4-dichlorophenyl)-urea and N-(3,4-dichlorophenyl)-N'-methyl urea, as well as some 3,4-dichloroaniline and 3,4-dichlorophenol (Hodge et al., 1967).

Propanil was readily metabolized by rainbow trout in the present study, forming at least ten metabolites. It was also readily eliminated as parent compound. One metabolite recovered from trout bile was identified by GC/MS as either 3',4'-dichloro-2-hydroxypropionanilide or 3',4'-dichloro-3-hydroxypropionanilide. In mammals, propanil has been reported to be hydrolyzed to 3,4-dichloroaniline by an acylamidase enzyme (Williams and Jacobson, 1966). Other mammalian studies tend to confirm this (Singleton and Murphy, 1973; Chow and Murphy, 1975). However, in the present study, 3,4-dichloroaniline formation from propanil could not be consistently demonstrated.

Herbicide Stability in Lake Superior Water

Dinoseb concentrations in Lake Superior water declined by 21% in 40 days in the present study. In a study of dinoseb toxicity to cutthroat trout (Woodward, 1976), a solution of the chemical in test water that was aged for 4 weeks was slightly less toxic than an unaged solution. The 96 hr LC_{50} of the aged dinoseb solution was 87 $\mu g \cdot L^{-1}$, as compared to 71 $\mu g \cdot L^{-1}$ for the unaged solution. This would represent a decreased toxicity of about 22% in the aged solution. However, in both the present study and in the study by Woodward (1976), microbial metabolism may not have been as important as it would be in actual field situations. Lake Superior is a cold oligotrophic body of water, and Woodward used standard reconstituted deionized water.

Dinoseb is readily photolysable in aqueous solution in the laboratory

when irradiated by ultraviolet light with an absorption maximum at 375 nm wavelength, which includes that part of the solar ultraviolet that reaches the earth (Crosby and Li, 1969). Thus, photolysis may be important in the dissipation of dinoseb in the field.

Propanil had an estimated half-life of 65 days in the present study, with the principal breakdown product identified as 3,4-dichloroaniline. The dissipation of propanil in Lake Superior water was much slower than its dissipation in actual field studies (Deuel et al., 1977; Popova, 1973), possibly due to differences in the richness of the microbial communities.

Pure chemical hydrolysis is considered a minor route of propanil degradation as sterile buffered solutions were stable for more than 4 months (El-Dib and Aly, 1976a). Propanil is readily degraded by the microbial community in soil (Kearney et al., 1970), and 3,4-dichloroaniline has been identified as a microbial transformation product in soil and water (El-Dib and Aly, 1976b; Deuel et al., 1977; Sharabi and Bordeleau, 1969). Although propanil dissipated rapidly in field studies (Deuel et al., 1977; Popova, 1973), El-Dib and Aly (1976b) found that mixed microflora from river water and sewage did not utilize propanil over a 4 month period and that active degradation occurred only after the addition of a heavy inoculum of Bacillus cereus.

Bromacil and diuron remained stable in Lake Superior water in the present study. Bromacil declined in concentration by 6% over 40 days, while diuron concentrations changed by less than 1% over a 36 day period. Alachlor was not studied for its stability in water.

Under intense ultraviolet radiation in the laboratory, a 1 $mg \cdot L^{-1}$ solution of bromacil was completely decomposed in 10 minutes (Kearney et al., 1969). Under conditions of natural summer sunlight for 4 months and laboratory irradia-

tion which simulated natural sunlight conditions, bromacil was photo decomposed very slowly (Moilanen and Crosby, 1974), indicating that photo decomposition probably makes only a minor contribution to the environmental disappearance of bromacil, unless in the presence of natural photosensitizers. Indeed, the addition of certain photosensitizers to bromacil solutions has since been shown to result in photodecomposition by sunlight (Acher and Saltzman, 1980).

Diuron concentrations remained stable for more than 4 months in a laboratory study of hydrolysis rate conducted at 20°C and at pH values of 5, 7, and 9 (El-Dib and Aly, 1976a). They stated that in the pH range of most natural surface waters (pH 6 to 9), the phenylamide herbicides (including diuron) would be expected to persist for long periods, and that pure chemical hydrolysis in the aquatic environment would be a minor route of degradation. Field and laboratory studies indicate that diuron is moderately persistent in the aquatic environment.

Alachlor was rapidly degraded in a model ecosystem study (Yu et al., 1975). Of the total radioactivity present in water after 33 days, only 1.8% was parent alachlor with the remainder consisting of at least 8 degradation products. However, information from field studies is needed to determine if alachlor is also rapidly degraded under actual field conditions.

Tetrachloroazobenzene (TCAB) as Contaminant of Propanil

TCAB has been identified as a contaminant of commercial propanil at concentrations up to 2.9 $mg \cdot g^{-1}$ (Bunce et al., 1979), and in diuron at concentrations up to 28 $\mu g \cdot g^{-1}$ (Sundstrom et al., 1978; Hill et al., 1981). TCAOB has been reported as present in diuron formulations at concentrations of 1-2 $\mu g \cdot g^{-1}$ (Sundstrom et al., 1978). TCAB was present in the technical propanil used at a concentration of 0.67 $mg \cdot g^{-1}$. No TCAOB was detected. Neither

contaminant was detected in the diuron used here.

Both TCAB and TCAOB are formed during the production of 3,4-dichloroaniline or during its further conversion to herbicides (Poland et al., 1976). TCAB residues have been detected in soils treated with propanil (Poland et al., 1976). These residues may have arisen either from the commercial formulation itself (Bunce et al., 1979) or as microbial transformation products (Bartha et al., 1968; Chisaka and Kearney, 1970; Bourdeleau et al., 1972; Lay and Ilnicki, 1974). TCAOB has also been found in soil as a microbial degradation product of propanil (Still and Herrett, 1976; Kaufman et al., 1972).

TCAB is approximately isosteric to 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran, two extremely potent toxins and teratogens (Hsia and Kreamer, 1979a; Hsia et al., 1977). All three compounds were potent inducers of hepatic aryl hydrocarbon hydroxylase (AHH) activity (Poland et al., 1976). TCAB was found to be weakly mutagenic by the Salmonella microsome test and to be toxic to mammalian cells in vitro (Hsia et al., 1977). TCAB induced unscheduled DNA synthesis in rat hepatocytes in vitro (Hsia and Kreamer, 1979b), and was considered a potential carcinogen. Hsia and Kreamer (1979a) found that the potency of TCAB and TCAOB as microsomal enzyme inducers in in vivo exposures with rats surpassed that of mirex and kepone, which were studied by Kaminsky et al. (1978) under comparable conditions. TCAOB was a more potent inducer of cytochrome P-448 than TCAB (Hsia and Kreamer, 1979a). TCAB and TCAOB exposures in rats have resulted in liver hypertrophy, histopathological alterations of hepatocytes, and induction of mitogenesis (Schrankel et al., 1980).

The significance to aquatic organisms of the presence of trace amounts of TCAB and TCAOB in water is not known. Deuel et al., (1977) found only

trace quantities of TCAB approximating the detection limit of 0.2 $\mu g \cdot L^{-1}$ in rice paddy water 24 hrs after flooding in 1973, and none at longer time intervals after flooding. No TCAB was detected in water samples collected up to 24 hrs after flooding in 1974 or 1975. Teratogenic effects were observed in fathead minnow fry of the present study at 3.8 $\mu g \cdot L^{-1}$ of propanil, but whether these effects were due to propanil itself, to the TCAB contaminant, or to a combination of these compounds is not known. TCAB would have been present at an extremely low concentration at a propanil exposure of 3.8 $\mu g \cdot L^{-1}$, approximating a concentration in the water of 2.5 $ng \cdot L^{-1}$. However, single injections of 10 ng TCAB into chicken eggs resulted in severe edema in the embryos (Schrankel et al., 1982).

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APPENDIX A

CHEMICAL CHARACTERISTICS OF THE TOXICITY TEST WATER.

TABLE A-1. MEANS, STANDARD DEVIATIONS AND RANGES OF DISSOLVED OXYGEN CONCENTRATIONS (PERCENT OF SATURATION) IN CONTROL AND EXPOSURE CHAMBERS FOR FATHEAD MINNOWS (Pimephales promelas) EXPOSED TO FIVE HERBICIDES IN ACUTE AND EARLY LIFE-STAGE TESTS

Herbicide		Acute Tests			Early Life-Stage Tests		
		N	Mean ± S.D.	Range	N	Mean ± S.D.	Range
Alachlor	Control	3	98.6 ± 0.4	97.3-98.5	11	79.3 ± 14.9	44.2-95.7
	Exposures	5	98.0 ± 0.6	98.3-99.3	2	76.4 ± 10.6	68.8-83.3
Bromacil	Control	3	96.3 ± 0.2	96.1-96.5	5	88.8 ± 5.7	81.9-95.4
	Exposures	6	96.1 ± 0.4	95.5-96.6	11	83.7 ± 11.7	52.2-92.5
Dinoseb	Control	3	96.1 ± 3.2	92.8-99.2	5	90.3 ± 6.3	82.1-99.5
	Exposures	6	95.8 ± 2.3	92.0-98.9	6	87.3 ± 3.0	82.2-91.4
Diuron	Control	2	90.1 ± 2.1	88.6-91.6	5	90.7 ± 1.6	88.3-92.6
	Exposures	2	92.0 ± 3.5	89.6-94.5	6	91.9 ± 1.1	89.9-92.9
Propanil	Control	3	91.6 ± 0.5	91.2-92.2	7	79.8 ± 9.6	66.9-92.1
	Exposures	5	90.0 ± 1.4	88.1-91.3	2	73.7 ± 28.9	53.2-94.1

TABLE A-2. SOME WATER QUALITY CHARACTERISTICS DURING ACUTE EXPOSURES OF FATHEAD MINNOWS (Pimephales promelas) TO HERBICIDES. VALUES ARE MEANS ± S.D.

Herbicide	N	pH	Total alkalinity (mg/l as $CaCO_3$)	Total hardness (mg/l as $CaCO_3$)	Total acidity (mg/l as $CaCO_3$)
Alachlor	4	Nd*	40.9 ± 0.3	43.7 ± 1.0	2.0 ± 0.4
Bromacil	2	7.4 ± 0.0	42.1 ± 0.3	49.4 ± 0.3	2.0 ± 0.0
Dinoseb	3	7.6 ± 0.3	45.6 ± 0.4	49.0 ± 0.9	2.0 ± 0.0
Diuron	3	7.5 ± 0.1	42.1 ± 2.0	46.4 ± 2.2	2.0 ± 0.0
Propanil	3	7.5 ± 0.0	42.1 ± 0.1	47.2 ± 1.4	2.0 ± 0.6

* No determinations.

TABLE A-3. SOME WATER QUALITY CHARACTERISTICS DURING EARLY LIFE-STAGE EXPOSURES OF FATHEAD MINNOWS (Pimephales promelas) TO HERBICIDES. VALUES ARE MEANS ± S.D.

Herbicide	N	pH	Total alkalinity (mg/l as $CaCO_3$)	Total hardness (mg/l as $CaCO_3$)	Total acidity (mg/l as $CaCO_3$)
Alachlor	7-8	7.4 ± 0.1	40.8 ± 3.9	47.1 ± 2.0	2.0 ± 0.2
Bromacil	3	7.4 ± 0.1	42.0 ± 0.5	50.6 ± 0.7	1.9 ± 0.2
Dinoseb	7	7.5 ± 0.1	45.9 ± 0.4	49.7 ± 1.0	1.9 ± 0.0
Diuron	5	7.5 ± 0.1	46.9 ± 2.9	48.4 ± 4.3	1.6 ± 0.4
Propanil	5-6	7.2 ± 0.4	41.7 ± 1.6	49.1 ± 2.0	2.0 ± 0.1

APPENDIX B

MORTALITY CURVES FOR ACUTE FLOW-THROUGH TOXICITY TESTS

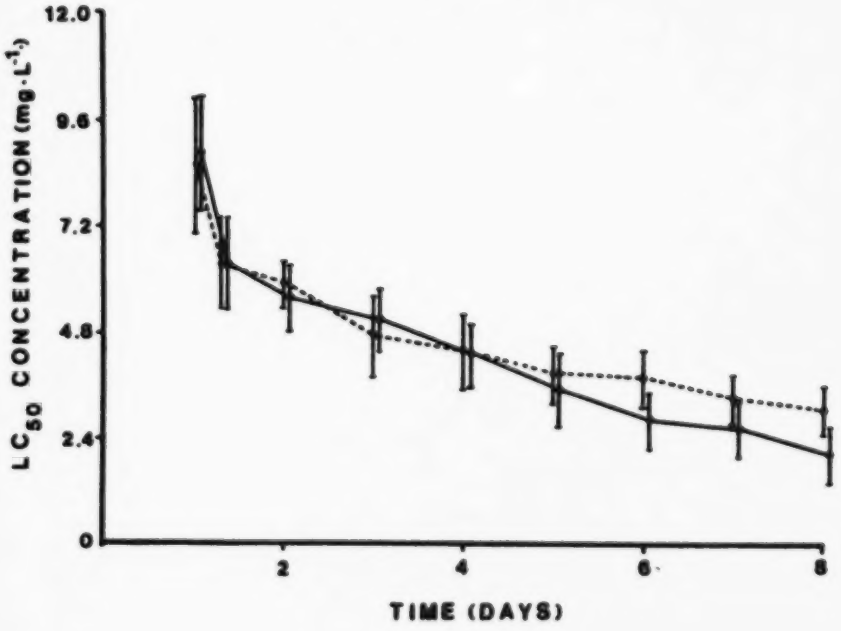

Figure B-1. LC_{50} concentrations for duplicate alachlor toxicity tests with 30-day old fathead minnows (Pimephales promelas).

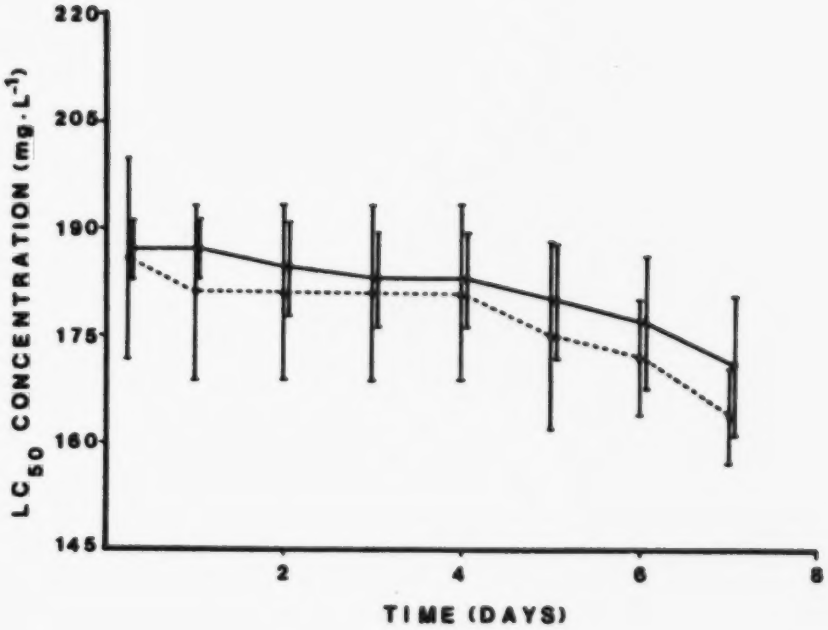

Figure B-2. LC_{50} concentrations for duplicate bromacil toxicity tests with 30-day old fathead minnows (Pimephales promelas).

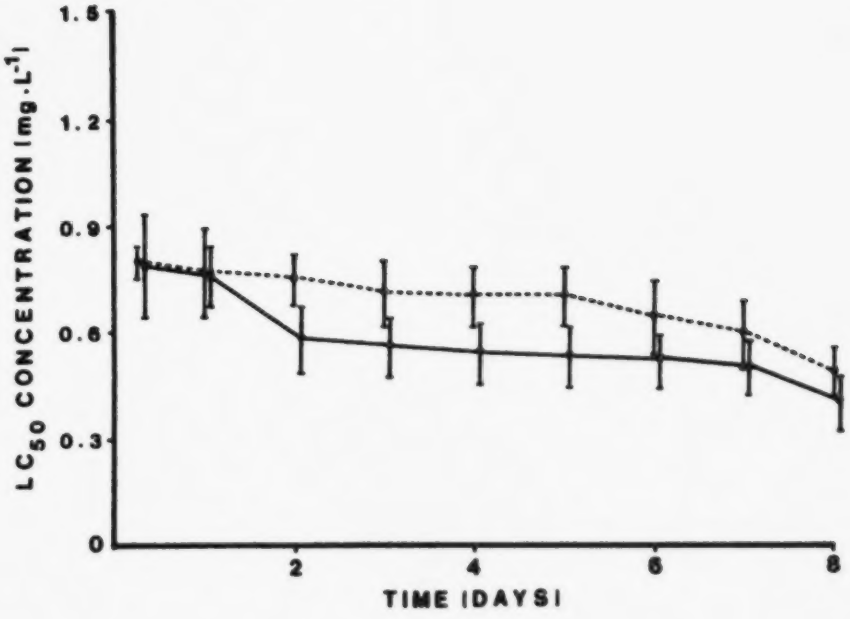

Figure B-3. LC_{50} concentrations for duplicate dinoseb toxicity tests with 30-day old fathead minnows (Pimephales promelas).

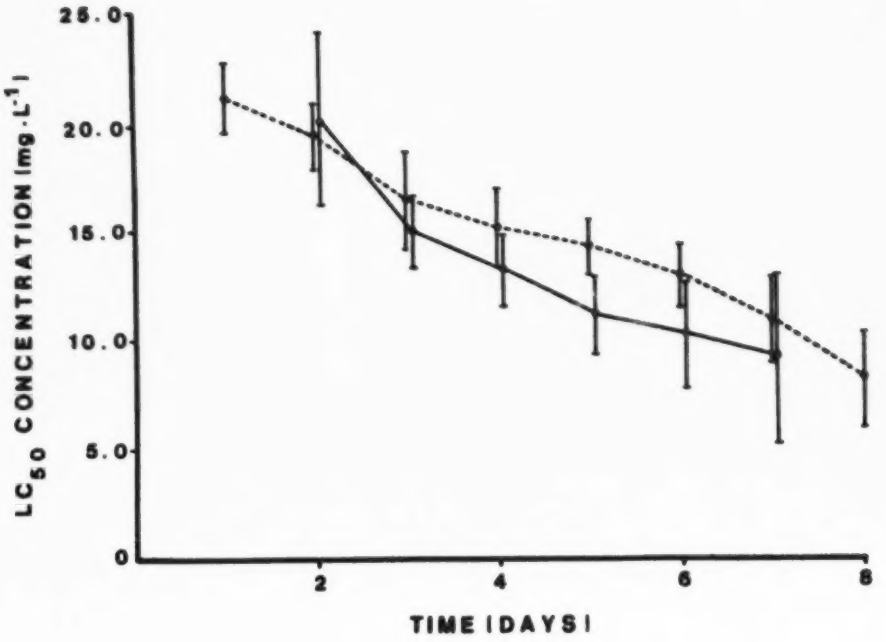

Figure B-4. LC_{50} concentrations for duplicate diuron toxicity tests with 30-day old fathead minnows (Pimephales promelas).

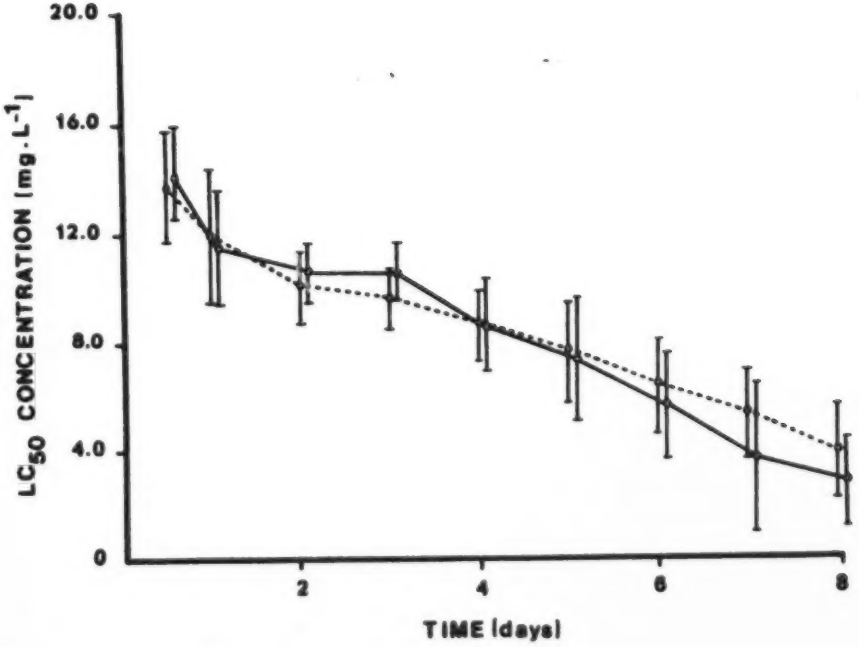

Figure B-5. LC_{50} concentrations for duplicate propanil toxicity tests with 30-day old fathead minnows (Pimephales promelas).

APPENDIX C

WATER CONCENTRATIONS AND WHOLE FISH TISSUE CONCENTRATIONS OF TOTAL ^{14}C FROM HERBICIDE BIOCONCENTRATION STUDIES WITH THE FATHEAD MINNOW (Pimephales promelas)

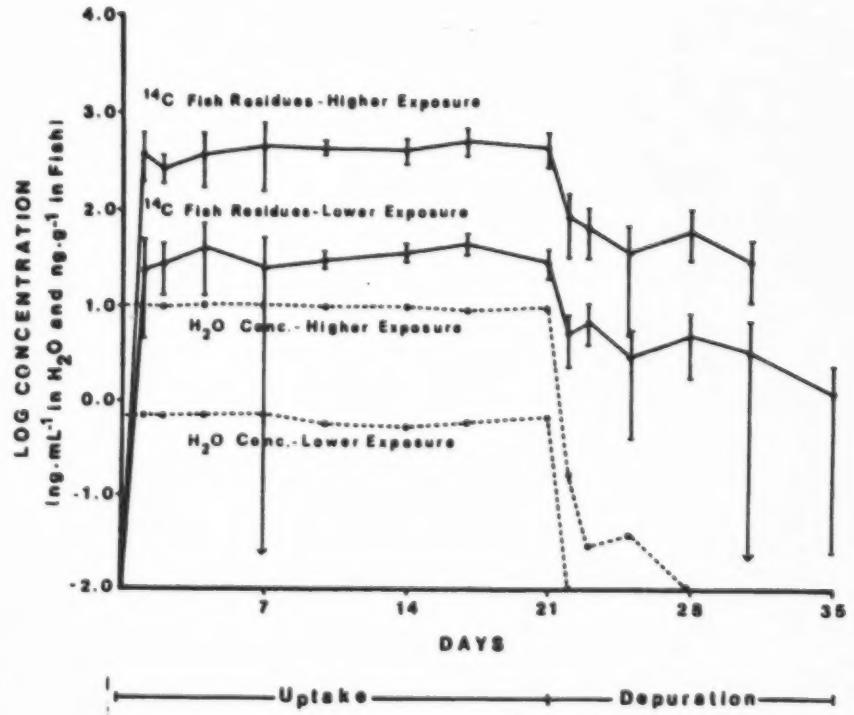

Figure C-1. Log mean exposure water concentrations of ^{14}C-labeled alachlor ($ng \cdot mL^{-1}$) and log mean (± S.D.) whole fish total ^{14}C residues ($ng \cdot g^{-1}$) during uptake and depuration phases.

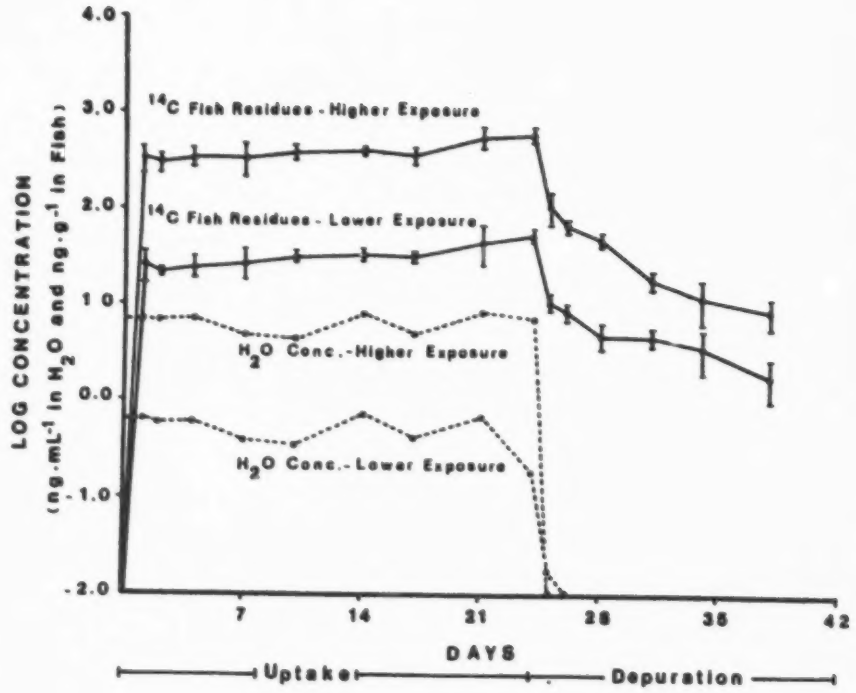

Figure C-2. Log mean exposure water concentrations of ^{14}C-labeled dinoseb ($ng \cdot mL^{-1}$) and log mean (± S.D.) whole fish tissue ^{14}C residues ($ng \cdot g^{-1}$) during uptake and depuration phases.

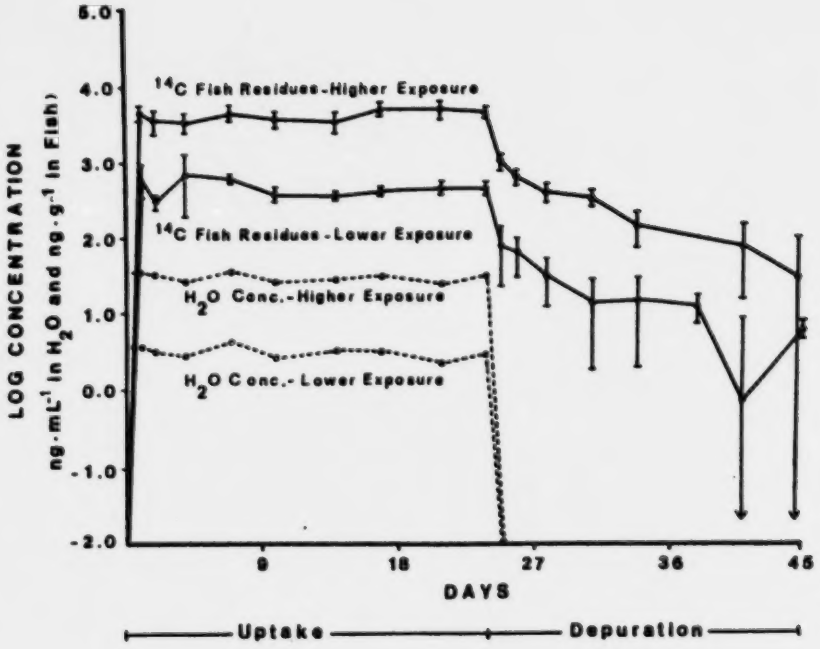

Figure C-3. Log mean exposure water concentration of ^{14}C-labeled diuron ($ng \cdot mL^{-1}$) and log mean ($\pm$ S.D.) whole fish total ^{14}C residues ($ng \cdot g^{-1}$) during uptake and depuration phases.

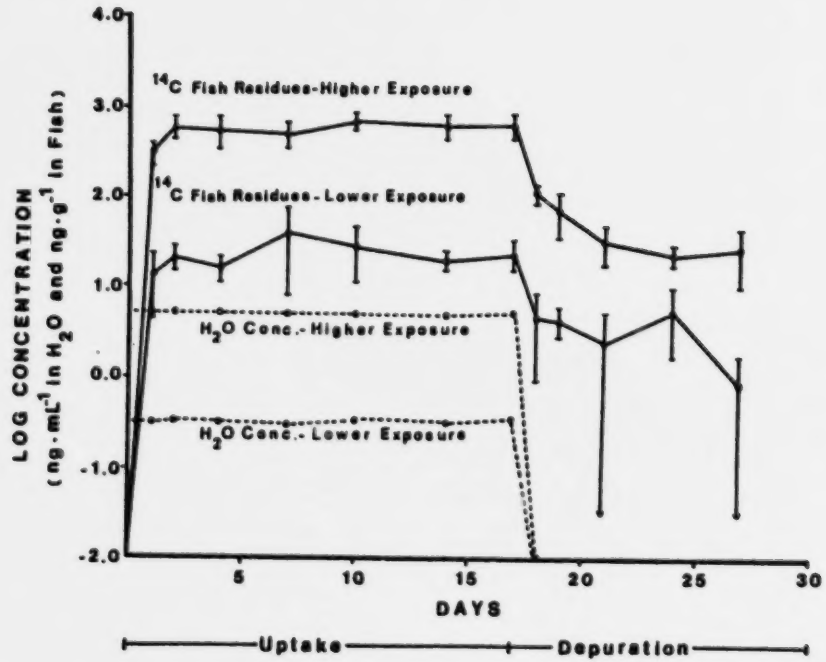

Figure C-4. Log mean exposure water concentrations of ^{14}C-labeled propanil ($ng \cdot mL^{-1}$) and log mean (± S.D.) whole fish total ^{14}C residues ($ng \cdot g^{-1}$) during uptake and depuration phases.

APPENDIX D

MASS CHROMATOGRAMS AND RECONSTRUCTED ION CHROMATOGRAMS FOR STANDARDS OF 3,3',4,4'-TETRACHLOROAZOBENZENE (TCAB), 3,3,'4,4'-TETRACHLOROAZOXYBENZENE (TCAOB), p-DIIODOBENZENE (DIB), AND TECHNICAL GRADES OF PROPANIL AND DIURON

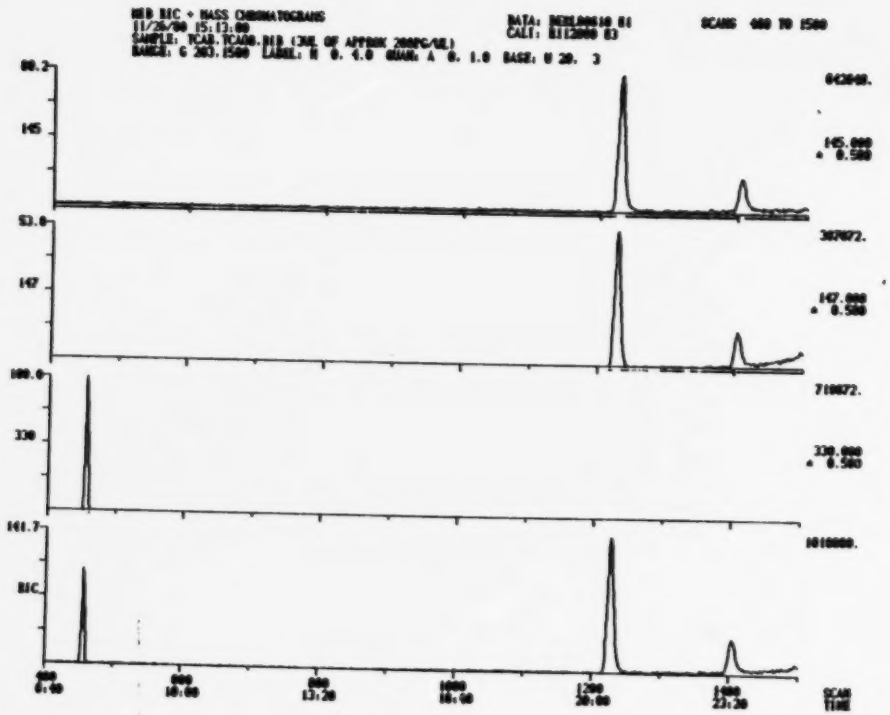

Figure D-1. Mass chromatograms and reconstructed ion chromatogram (RIC) for standards of 3,3',4,4'-tetrachloroazobenzene (TCAB), 3,3',4,4'-tetrachloroazoxybenzene (TCAOB), and p-diiodobenzene (DIB).

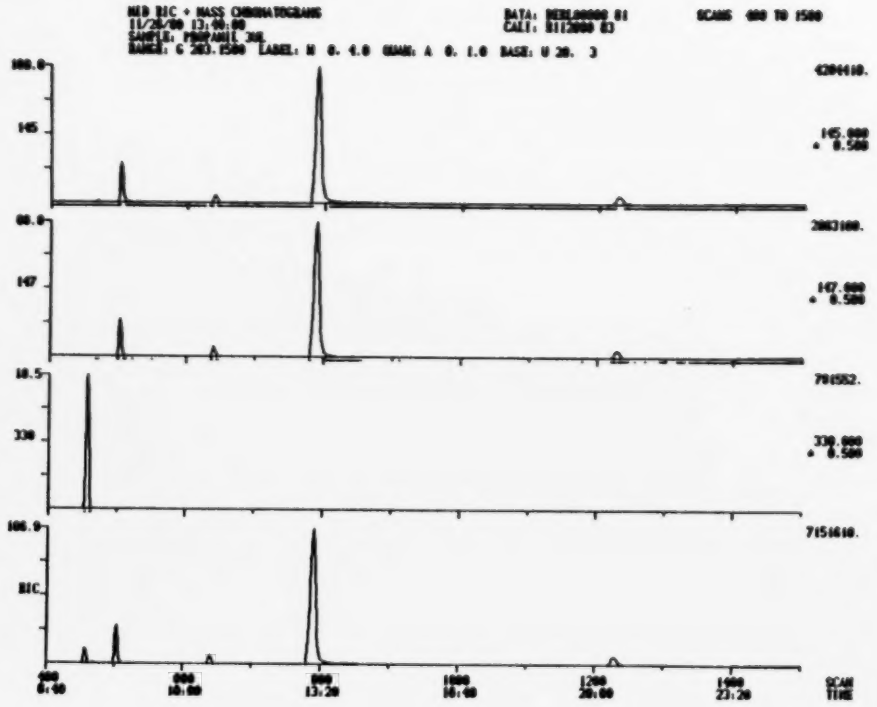

Figure D-2. Mass chromatograms and reconstructed ion chromatogram (RIC) of technical grade propanil monitored for 3,3',4,4'-tetrachloroazobenzene (TCAB) and 3,3',4,4'-tetrachloroazoxybenzene (TCAOB).

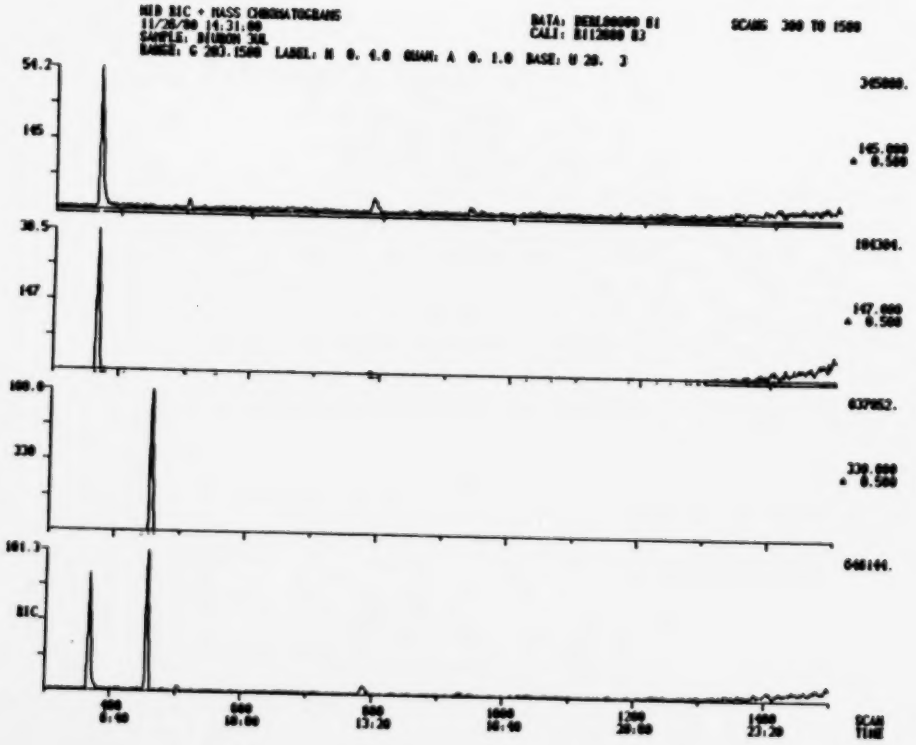

Figure D-3. Mass chromatograms and reconstructed ion chromatogram (RIC) of technical grade diuron monitored for 3,3',4,4'-tetrachloroazobenzene (TCAB) and 3,3',4,4'-tetrachloroazoxybenzene (TCAOB).